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THE FORMATION OF THE FIRST POLAR SPINDLE IN THE EGG OF BUFO LENTIGINOSUS.

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In a previous paper on "The Maturation and Fertilization of the Egg of *Bufo lentiginosus*" (King, 10), the formation of the first polar spindle and the subsequent divisions of the chromosomes were very incompletely described owing to a lack of material showing the details of these processes. During the spring of 1899, a large number of toads were collected soon after they had emerged from their hibernation, and from three of them sufficient material was obtained to give a more complete history of the late maturation processes. A short account of my study of this period in the development of the egg has already appeared (11); a detailed account is given in the present paper.

I. Material and Methods.

As soon as possible after the toads were captured they were killed by pithing and the body opened at once to ascertain the condition of the ovaries. In a great majority of cases the eggs were found free in the cœlomic cavity and were, therefore, of no use for the purpose intended, as previous investigations had shown that eggs which have broken through the wall of the ovary invariably contain a fully formed maturation spindle lying at the periphery near the center of the black hemisphere.

In several instances, all of the eggs were still attached to the walls of the ovaries when the toad was killed. In these cases some of the eggs were put at once into a dish of fresh spring water and the rest were left in the body of the female which was

kept in a moist chamber. A few eggs from each of these series were then fixed at intervals of ten minutes for a period of several hours. By opening an egg under a dissecting lens after it has been taken from the fixing solution and put into 50 per cent. alcohol, one can tell definitely whether the late maturation processes have begun or not; for, if the nuclear membrane in still intact, the nucleus retains its rounded form and can be readily separated from the rest of the egg contents. If one hour after the toad is killed, an examination of freshly fixed eggs shows that the nucleus is still intact, the entire set of eggs can be discarded, as it has been found that further development does not take place unless the germinal vesicle breaks down previous to this time, although the eggs, whether kept in water or in the body of the female, show no signs of disintegration for many hours.

In one case the germinal vesicle was just breaking down when the eggs were first examined under the dissecting lens; in another set of eggs the germinal vesicle could no longer be dissected out half an hour after the toad was killed. These two lots of eggs gave overlapping series of stages which corresponded in every respect. A third set of eggs showed no signs of the germinal vesicle when first examined, and when sectioned showed maturation processes identical with those taking place in eggs which had been developing in water for several hours.

In all these three sets of eggs, the first polar body was given off in the normal position and apparently in the normal manner before the eggs showed any signs of disintegration. No difference was noticed in the development of eggs which had been put into water and those which had been left in the body of the toad. It does not seem, therefore, that such unusual conditions interfere at all with the late maturation processes provided these processes have started before the normal conditions are changed. No attempt was made to fertilize these eggs artificially, as it has never been found possible to fertilize either the eggs of *Bufo* or of *Rana* until they have received the thick jelly-like membrane which is secreted around them in the oviducts.

In all cases the eggs were fixed in corrosive-acetic and stained with a combination stain of borax carmine and Lyon's blue as described in a previous paper (King, 10).

II. The Disintegration of the Germinal Vesicle.

I have already given in detail a description of the early stages in the breaking down of the germinal vesicle, and as this new material confirms but adds nothing to that description, it will be necessary to give only a brief account of the changes in the egg directly preceding the formation of the spindle.

At the end of the hibernation period the germinal vesicle lies in the upper hemisphere of the egg. It is round in outline and contains a large number of nucleoli which usually form a ring enclosing the chromatin threads. A layer of granular substance staining differently from the cytoplasm, surrounds the lower pole of the germinal vesicle and extends half way up each side. This substance appears homogeneous at first and then becomes a compact, fibrous band of uniform thickness. I have called this band a "line of radiation," because, as soon as the nuclear membrane has disappeared in this region, the karyoplasm of the nucleus forms into coarse granules and a pronounced radiation extends up into the nuclear substance from the entire length of the fibrous band below. The karyoplasmic granules soon become smaller and more numerous and finally disappear entirely, while the radiation from below continues to increase and often extends nearly to the upper surface of the egg. The rays forming this radiation are very fine, and their outer ends run, apparently, into the coarse network which comes to fill the entire space formally occupied by the germinal vesicle. During these changes, the nucleoli have lost their power of staining and have begun to disintegrate.

When the nuclear membrane breaks down, twenty-four chromosomes, arranged in pairs, are scattered throughout the upper part of the nuclear space. The ends of each pair then unite to form a closed ring near which a small aster usually appears. The aster has no centrosome and its rays rarely touch the chromatin ring. At the next stage, when the radiation from below has reached its greatest extent, the asters and the chromatin rings entirely disappear. Later, when the radiation has begun to decrease, a large number of small round chromatin granules are found near or on the line of radiation which has been gradually shortening during this period. When the chromatin granules

first appear they stain very faintly, but they soon take the deep carmine stain characteristic of chromatin, and then fuse into several large, irregular clumps.

III. The Formation of the First Polar Spindle.

The line of radiation, shortly after the appearance of the chromatin granules, is shown in Fig. 1. It is a short, fibrous band with its ends, usually, though not invariably, slightly curved in towards the center of the egg. This structure, which is to become the first polar spindle, lies some distance below the surface of the egg in a small accumulation of granular substance formed, possibly, from the karyoplasm of the germinal vesicle. Its longitudinal axis may be either parallel or oblique to the surface of the egg, the latter position being the more common. Running out in every direction from the compact meshwork of fibers are numerous fine, thread-like rays which are longest and most numerous at the middle of the forming spindle where they extend out between the yolk spherules and seem to be continuous with the cytoplasmic network of the egg.

Collected near the middle of the spindle is a mass of small chromatin granules which are of uniform size and stain but faintly in comparison with the chromosomes of an earlier and of a later period. There is a very large number of these granules and it is quite impossible to count them satisfactorily; two other sections of the same egg each show as many granules as are shown in Fig. 1.

The nucleoli from the germinal vesicle appear at this period as irregular, yellowish green, refractive bodies which are scattered throughout the upper hemisphere of the egg, often lying quite close to the spindle. They disappear at different times in different eggs. Sometimes they have all been absorbed before the chromosomes have divided; sometimes they can still be found after the first polar body has been given off. I have never found any traces of them, however, after the spermatozoön has entered the egg.

Not more than fifteen minutes after the stage of Fig. 1, the chromatin granules begin to fuse into irregular-shaped clumps. The number and size of these clumps vary greatly in different eggs, in some cases there are but four or five of them, in others

at least twenty. Owing, probably, to their greater volume, these larger masses always stain much more intensely than do the small granules. Meanwhile the spindle has lost its uniform diameter and has become much thicker in the middle where the meshwork of fibers appears more distinct and more regular. The spindle soon becomes barrel-shaped and its fibers are quite clearly defined in the middle region but not at the poles (Fig. 2). The radiation from the spindle disappears entirely except at the poles where it forms distinct asters; some of the rays are very long and cross each other at the equator of the spindle. During its migration towards the upper pole of the egg the spindle shortens somewhat and gradually becomes more slender and pointed, a phenomenon seen by Van Name (17) in the eggs of Planarians, by Korschelt (12) in *Ophryotrocha*, by Griffin (8) in *Thalassema*, and by Boveri (1) in *Ascaris*.

At no stage in the formation of the spindle or in its later history can any centrosome be found in the polar asters. As the spindle becomes more pointed, the rays converge more sharply at the poles, but even when the radial systems are best developed (Figs. 2, 3), the rays appear to run into each other in the center of each aster and there is not the slightest trace of any kind of a central body. Carnoy and Lebrun (2) in their study of the batrachian egg, Eismond (5) in his work on *Siredon* and *Triton*, Fick (6) in studying the maturation of the Axolotl egg, and *Sobotta* (15, 16) in working on the egg of the mouse and of *Amphioxus*, have all failed to find a centrosome in the asters of the polar spindles. If such a structure is normal in these eggs and also in the egg of *Bufo lentiginosus*, methods of fixation and staining which have so clearly demonstrated its presence in other eggs are totally inadequate in these cases to show the slightest trace of it.

At the stage of Fig. 3, the small chromatin granules have entirely disappeared. Whether they have all gone into the large chromatin clumps or whether some have been absorbed by the cytoplasm cannot be determined. At this time the number of large chromatin masses still varies slightly in different eggs; in some cases there are nine such clumps of chromatin, in others at least fifteen. These chromatin masses are, for the most part, scattered irregularly along the spindle fibers, occasionally, however, one or more of them can be seen entirely outside of the

spindle (Fig. 4, *CM*). Isolated masses of chromatin are sometimes found near the spindle at a much later period when the chromosomes are at the equator preparing to divide. They have entirely disappeared by the time the first polar body is given off, possibly serving as food for the cytoplasm as suggested by Gardiner (7).

During the next half-hour, the irregular chromatin masses change into chromosomes with a definite shape. The change does not take place at the same time in all of the chromatin clumps; in fact, until the chromosomes are arranged at the equator of the spindle ready to divide, they may be found in several different stages of development on the same spindle. Twelve chromosomes, one-half the number characteristic of the somatic cells of this species differentiate from the chromatin masses. The chromosomes are scattered over the entire spindle and are at first somewhat triangular in shape (Fig. 3), later they become rod-shaped structures which may lie with their long axis parallel, oblique, or even at right angles to the longitudinal axis of the spindle (Fig. 4). Sooner or later, however, the long axis of each chromosome comes to lie parallel with the spindle fibers and the chromosomes then have a rounded knob in the middle region and frequently also a smaller knob at each end (Figs. 4, 5). Later the middle knob becomes more prominent and the end knobs disappear (Fig. 5).

At the stage of Figs. 2–3 the asters at the spindle poles reach their greatest development. There are many long rays from each aster which run nearly parallel with the spindle fibers and cross each other at the equator of the spindle, and fewer and much shorter rays going out in other directions. Soon after this time the asters begin to degenerate. The shorter rays disappear first and by the time the spindle has reached the periphery of the egg there is not a trace of the radiation left. The spindle fibers then converge at the poles which are surrounded by a small accumulation of granular substance probably formed from the disintegrated rays (Fig. 7).

There is often a marked difference in the size of the chromosomes on the same spindle even when they are of exactly the same shape. One or two of the chromosomes may extend over one-third the length of the spindle, the others being not more

than one-half as large (Fig. 5). This difference is not found at a later period; for, when the chromosomes are arranged at the equator of the spindle ready to divide, they are considerably smaller than the chromosomes of an earlier period and are all, apparently, of the same size.

While the chromosomes are being arranged at the equator of the spindle they undergo further changes in form. The polar arms shorten considerably, while the thick knob at the middle increases in size and gradually spreads out laterally, thus forming two wing-like projections on the chromosomes (Figs. 6, 7). In proportion as the lateral wings grow larger the polar arms of the chromosomes become shorter and thinner, so that there can be no question but that this lateral growth takes place at the expense of the rest of the chromosome. In a dorsal view, the wings appear to be spread out flat on the spindle and the chromosome has the appearance of a cross in which the polar arms are somewhat longer than the equatorial arms (Fig. 6). In a lateral view, however, the wings are seen to be raised up from the spindle while the polar arms are extended along the spindle fibers. Carnoy and Lebrun (2) have applied the term "oiselet" to this stage in the development of the chromosome. The typical oiselet stage is followed by one in which the body of the "bird" gradually disappears while the wings constantly increase in size (Fig. 7). Very soon, all that is left of the original polar arms is a slight projection on each side of the angle formed by the meeting of the two wings (Fig. 8). In the succeeding stage every trace of the polar arms has disappeared and there are twelve broad V-shaped chromosomes arranged at the equatorial plate with the angle of the V turned in towards the center of the spindle (Fig. 9). Usually, before this last stage is reached, the spindle has come to lie close to the surface of the egg and nearly radial in position. This is by no means invariably the case, however, as sometimes the spindle is still some distance below the surface of the egg when the chromosomes have divided in preparation for the first maturation division.

Fig. 6 shows part of a section of an egg fixed as soon as possible after the toad was killed. The spindle lies at the periphery of the egg and the chromosomes, with well-developed lateral wings, are at the equator. That this egg and others from the

same series are normal cannot be questioned. They show phenomena exactly similar to those seen in eggs that have been developing in water for some three hours, and leave no doubt but that the earlier processes described above are normal in spite of the unusual conditions to which many of the eggs were subjected.

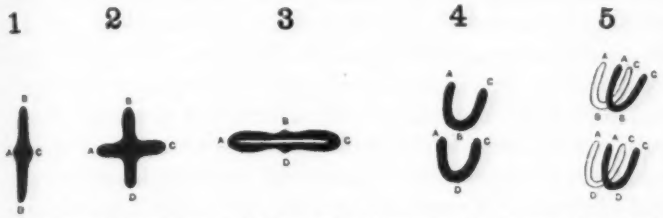
Four V-shaped chromosomes in which all traces of the polar arms have disappeared are shown in Fig. 9. The arms of the V's are broad flat plates which form a sharp acute angle with each other. There is, in this case, no sign of a splitting in any of the chromosomes which are all of the same size and shape and arranged at the equatorial plate with the angle of the V turned in towards the center of the spindle, a characteristic arrangement of the chromosomes at this period. An equatorial section of a spindle in the same stage as Fig. 9, is seen in Fig. 10, where all twelve chromosomes are present. In this egg there are also found near the spindle a number of nucleoli which are in the process of disintegration.

Usually the first indication of any division of the chromosomes is seen at the stage of Fig. 11 when the polar arms have entirely disappeared and the chromosomes are broad V-shaped structures. At this time the ends of the V's often show a deep indentation (Fig. 11) indicating the longitudinal splitting of the chromosomes. Occasionally I have found the first division coming in at an earlier period before the entire disappearance of the polar arms. Such a division is seen in the chromosome at the left in Fig. 7. In all such cases the splitting is confined entirely to the lateral wings and never extends into the polar arms.

In the egg from which Fig. 12 was drawn, there are twenty-four V-shaped chromosomes which are similar to the twelve chromosomes in Fig. 10 in every respect except that they are much narrower. They have been produced, I believe, by a longitudinal division of the broad V-shaped chromosomes found at an earlier period. In some of the chromosomes shown in Fig. 12, the division for the second polar mitosis is seen. This second division of the chromosomes is not visible, at this stage, except in equatorial sections of the spindle. In longitudinal sections of the spindle the chromosomes always appear to be arranged in tetrad groups, one of which may be seen in Fig. 12. Such a group is, in reality, a pair of V-shaped chromosomes with

the angle of each V turned in towards the center of the spindle, the four ends of a pair of chromosomes projecting from the spindle give the appearance of a typical tetrad. The maturation divisions of the chromosomes are represented diagrammatically by text-figures 1–5.

In my previous paper, three sections from one egg (Figs. 25, 26, 27) were given in which the fully formed spindle lay some



Diagrams of the maturation divisions of the chromosomes in the egg of Bufo lentiginosus.

distance below the surface of the egg and the chromosomes were in the form of closed rings which were split longitudinally. This egg was undoubtedly abnormal and led to the wrong inference that these chromatin rings were identical with those found in the germinal vesicle just previous to its disintegration. If the split V-shaped chromosomes of Fig. 11 were to be spread out in the form of a ring and the second maturation division to take place before the halves of the ring separated, then exactly the same effect would be produced as previously illustrated in Figs. 25–27. I can only interpret the ring-shaped chromosomes in this abnormal egg — the one abnormality I have found in many hundreds of eggs sectioned — as due to a delay in the separation of the parts after the two divisions of the chromosomes had taken place.

According to Carnoy and Lebrun (2, 3) who have published a series of memoirs dealing with the development of the germinal vesicle and the formation of the polar bodies in the eggs of various Batrachia, the chromatin filaments in the egg of Salamander, *Alytes*, *Triton*, *Bufo* and *Rana* arise from repeated resolutions of the nucleoli in the germinal vesicle. As my own work on *Bufo* began with the fully formed egg taken from the animal just before the beginning of the hibernation period, I have not yet seen this

resolution of the nucleoli into chromatin threads. In all the eggs that I have examined in which the germinal vesicle was intact, the chromatin was always in the form of distinct chromosomes. These chromosomes had no connection whatever with the large round nucleoli which, with the combination stain used, always stain a deep blue while the chromatin invariably takes the carmine. I have frequently noticed, however, that many of the chromosomes end in small granules which take the same stain as the chromatin and that there are a number of similar granules scattered throughout the nucleus. Recent work on various forms has shown that unquestionably the term nucleolus has been applied to many different kinds of structures in the germinal vesicle. As a general term used to cover any definite structures in the germinal vesicle other than chromosomes, linin and karyoplasm, it may, perhaps, be fitly applied both to the large rounded structures (which I consider the only true nucleoli in the germinal vesicle) and to the smaller granules which stain like chromatin and which I believe to be chromatin that is not used for the chromosomes. The structures which, in my opinion, are the true nucleoli have nothing to do with the formation of the chromosomes for the first polar spindle, as they are never connected with the chromosomes in any way and can be traced step by step until they are absorbed by the cytoplasm of the egg after the spindle is completely formed.

Many of Carnoy and Lebrun's illustrations of the formation of the first polar spindle in the egg of *Bufo vulgaris* are strikingly like my own, but we differ somewhat in our interpretation of them. According to their view, when the germinal vesicle in the egg of *Bufo vulgaris* migrates towards the upper pole, and before the nuclear membrane disappears, the paired chromatin filaments (which are exactly like those I find in *Bufo lentiginosus* during the same period) break up into small granules which cannot be distinguished from the granules of karyoplasm. *All the nucleoli suffer the same fate as the chromosomes excepting about ten which remain to form the chromosomes of the first polar spindle.* The karyoplasm meanwhile, forms a pronounced radiation from the "plage fusoriale" at the lower pole of the germinal vesicle. "Les nucléoles prédestinés montent le long des fila-

ments" and are carried to the "plage fusoriale" where they either become vacuolated in the center and form a ring, or else they fuse into one large mass and later regain their individuality. When the spindle is first formed, the chromosomes are very irregular in shape and there are distinct asters at the spindle poles which never contain a centrosome.

In the egg of *Bufo lentiginosus*, I have traced the chromosomes of the germinal vesicle up to the stage where the ends of a pair of chromosomes unite to form a closed ring. After this time, although I have had an abundance of material and have searched very carefully through every section of the germinal vesicle in a large number of eggs, I have been unable to find any trace of the chromatin. There is, I believe, no doubt but that the chromatin rings break up into minute granules which may, possibly, be carried by the karyoplasmic radiation to the lower pole of the germinal vesicle where they later form the chromosomes of the first polar spindle. I have never seen anything in this egg, however, that would indicate that some of the *nucleoli* are destined to form the chromosomes of the first polar spindle. A large number of nucleoli are always present throughout the early stages of maturation and they all appear to be undergoing the same processes of disintegration. Carnoy and Lebrun might consider the large irregular masses shown in Fig. 2 to be nucleoli in the general sense in which they seem to use the word, but these masses have been formed by the fusion of smaller chromatin granules (Fig. 1) and are in no way connected with the true nucleoli of the germinal vesicle.

Carnoy and Lebrun have followed the details of the formation of the first polar spindle and the later changes of the chromosomes much more carefully in the egg of *Triton* than in any of the other amphibian eggs they have studied. Their account of this form agrees substantially with that of *Bufo vulgaris* as regards the breaking down of the germinal vesicle, with the important exception that in *Triton*, *all* of the nucleoli are absorbed by the cytoplasm, none of them are reserved, as in *Bufo Vulgaris*, to form the chromosomes of the first polar spindle. The chromatin threads which were resolved from the nucleoli at an earlier period, break up into very small granules when the membrane

of the germinal vesicle disappears. The twelve chromosomes which later arise *from a coalescence of the chromatin granules* are at first very irregular in shape and they are scattered all along the spindle fibers; subsequently they undergo a double longitudinal division in preparation for the giving off of the polar bodies. Any chromatin not used for the chromosomes is absorbed by the cytoplasm.

Although there are always twelve chromosomes on the first polar spindle in the egg of *Triton*, Carnoy and Lebrun find only 8–10 chromosomes in the equatorial plate of the first polar spindle in the egg of *Bufo vulgaris*, and but 4–5 chromosomes at each pole just previous to the giving off of the first polar body. The failure of these investigators to find the definite number of chromosomes that must be present unless the egg of *Bufo vulgaris* is a marked exception to the rule that the number of chromosomes is constant for a given species, may possibly be accounted for on the supposition that some of the chromosomes were lost when the eggs were sectioned or that the sections of the egg were made so thick that some of the chromosomes were not visible.

In a more recent paper, Lebrun (13) gives the results of a reexamination of the maturation processes in the egg of *Triton*. He states that the double longitudinal division of the chromosomes does not take place in the complicated manner previously described by Carnoy and Lebrun, but according to the scheme represented by my text-figures 1–5. The late maturation changes in the egg of *Triton* are, therefore, strikingly similar to those I have found taking place in the egg of *Bufo lentiginosus*. Lebrun still believes that in the eggs of *Rana temporaria* and of *Bufo vulgaris* a certain number of the nucleoli are reserved to form the chromosomes of the first maturation spindle. A reëxamination of the maturation stages in the eggs of these amphibians would probably show that in these forms also the chromosomes are derived from fused masses of chromatin granules and that they have no connection whatever with the true nucleoli.

I have examined a large number of the eggs of *Bufo* at the stages of Figs. 3–4 and I can see no reason for believing with Carnoy and Lebrun that a division of the chromosomes takes

place at this time. During this period the chromosomes are exceedingly varied in size and shape. If the chromosome is oblong, it may have either its long or its short axis parallel with the longitudinal axis of the spindle; if the chromosome is pyramidal in shape, either the base or the apex of the pyramid may rest on the spindle fibers. I regard all of the changes in the shape of the chromosomes up to the stage of Fig. 7 as due solely to a rearrangement of the chromatin material preparatory to the later divisions. The first indication of any division of the chromosomes is the longitudinal splitting of the lateral wings which in some few cases can be found before the disappearance of the polar arms (Fig. 7). The apparent separation of the lateral wings at *X*, Fig. 11, I consider to be due to the fact that the angle of the V-shaped chromosome was cut off in sectioning. It very frequently happens that portions of one or of several chromosomes on a spindle are removed in this way. Sometimes, as in Fig. 4, the median knob of a chromosome is lacking; sometimes, the lateral wings have been removed (Figs. 6, 7). In rare instances the cut off portion of the chromosome will be found in the next section of the egg; but as the chromosomes are quite small a careful examination of the following sections often fails to disclose the missing part.

As found to be the case in many eggs besides that of *Bufo*, for example in *Cerebratulus* (Coe), *Polychœrus caudatus* (Gardiner), *Thalassema* and *Zirphœa* (Griffin), and *Triton* (Carnoy and Lebrun), all the chromatin of the germinal vesicle does not go to form the chromosomes of the first polar spindle, some of it is thrown out into the cytoplasm where it degenerates and sooner or later completely disappears. Even in the segmentation stages of the egg of *Ascaris*, Boveri (1) found that some of the chromatin is thrown out of the nucleus and absorbed by the cytoplasm. In all these cases there is obviously a mass reduction of the chromatin in preparation for the succeeding division of the cell. It may be, as suggested by Gardiner, that "there are two kinds of chromatin stuff, the one insoluble and bearing the heredity which is to be transmitted to the daughter cells, and the other food for the cytoplasm." This theory would explain the facts as we now know them, but it cannot be proved until some stain can be found to differentiate the two sorts from each other.

Carnoy and Lebrun find a double division of the chromosomes in the egg of *Triton*, and they state that there is no reason why a longitudinal division of the chromosomes should not be a reduction division in the Weismann sense, in that it may separate the chromosome into two parts each containing different kinds of granules: it is certainly true if we admit a difference in the properties of the elementary granules. As all of the chromatin granules do not go into the chromosomes of the first polar spindle, there is a process of selection in the formation of the chromosomes and their subsequent division would be a permanent source of variation for the descendents.

The chromosomes of the first polar spindle in the egg of *Bufo lentiginosus* are at first exceedingly varied in shape; they may be round, triangular, or oblong. At this time it is obvious that they have no definite longitudinal axis. At the stage of Fig. 5 the chromosomes have elongated and lie parallel with the longitudinal axis of the spindle. When the wings have formed, there is a stage when the arms of the chromosomes are all approximately of the same length (Fig. 6). Is there a definite longitudinal axis at this time? If the part of the chromosome resting upon the spindle fibers is considered to be the longitudinal axis, then later this same axis is not only shorter than the transverse axis, but it practically disappears at the stage of Fig. 9. If shown Fig. 9 without the preceding figures, no one, I am sure would call the thickness of the chromosome at the angle of the V the longitudinal axis of the chromosome, and the division indicated in Fig. 11 would unhesitatingly be called a longitudinal division. If one arbitrarily states that the polar arms of the chromosomes in Fig. 5 form the true longitudinal axis, not only in this particular stage, but until division is completed, then the splitting seen in Fig. 11 is a transverse division, as is also the second division which takes place in the same direction. On the other hand, if the longer axis of the chromosome at the time when division occurs is considered to be the true longitudinal axis, then there is a double longitudinal division of the chromosomes and the egg of *Bufo* is thus brought into line with other amphibian eggs that have been studied. It would seem, as suggested by Sebaschnikoff (14), that the distinction between transverse

and longitudinal divisions of the chromosomes is not as important as many investigators have claimed: the division of the chromatin substance would appear to be the important thing, the manner of its achievement quite secondary, as Hertwig (9) has maintained.

There is, however, the following possibility to be considered. When the germinal vesicle breaks down, all of the chromosomes are arranged in pairs, in some cases the ends of a pair of chromosomes have united to form a closed ring. Very soon after this stage the chromosomes break up into granules and all traces of the chromatin substance is lost until innumerable chromatin granules appear in connection with the first polar spindle. It is conceivable that all of the chromatin granules belonging to a pair of chromosomes have remained united during this period of the apparent disintegration of the chromosomes, although I have not been able as yet to demonstrate such a union. If such is the case, then the chromosomes of the first polar spindle are bivalent structures, each being composed of the two chromosomes that had become paired at an earlier period of development. On this assumption it is probable that the knob-like thickening in the middle of the chromosomes, shown in Figs. 4 and 5, is caused by the fusion of the ends of the two chromosomes. In text-figure 1, *ABC* and *ACD* would represent the two chromosomes united at *AC*. The subsequent changes in the shape of the chromosomes serve merely to again elongate the original chromosomes (Text-fig. 3) which are finally separated by the division through *AC*. The first maturation division, therefore, is a reduction division and the second division only is a longitudinal one. It certainly cannot be mere chance that at the time of the breaking down of the germinal vesicle, the chromosomes should invariably become arranged in pairs. In light of the most recent investigations on spermatogenesis and ovogenesis it would seem as if the above explanation must be the true one for the maturation divisions in the egg of *Bufo*, although at present I am not able to prove it. I hope that the work I am doing on the spermatogenesis of this amphibian will throw some light on the maturation divisions in the egg.

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Explanation of Plate.

All figures were drawn with the aid of a camera lucida under a Zeiss Apoc. 2 mm., Oc. 4.

1. An early stage in the formation of the first polar spindle before the chromatin granules have fused into large masses.

2. A stage about one-half hour later than Fig. 1. The spindle has become barrel-shaped and the chromatin granules are fusing into large masses to form the chromosomes.

3. Twelve irregularly shaped chromosomes have differentiated from the chromatin masses and lie scattered along the spindle.

4. Spindle parallel to the surface of the egg. The chromosomes have elongated and many of them show a median knob. *C.M.*, chromatin mass outside the spindle.

5. About the same stage as Fig. 4. Chromosomes of very different sizes are found on the spindle.

6. Typical "oiselet" stage.

7. Chromosomes in various stages of development on the same spindle. In some of the chromosomes the splitting for the first maturation division can be seen while the polar arms are still present.

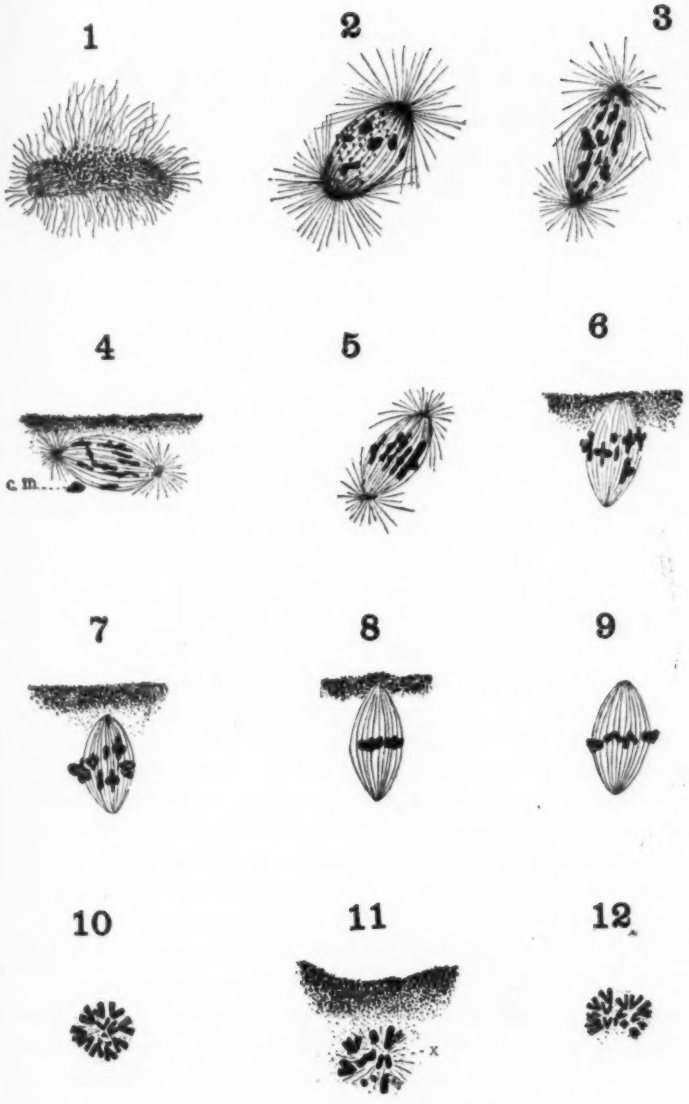
8. Section showing the growth of the lateral arms of the chromosomes at the expense of the polar arms.

9. The V-shaped chromosomes after the disappearance of the polar arms.

10. An equatorial section of a spindle at the stage of Fig. 9. All twelve chromosomes are present.

11. Equatorial section. The notched ends of some of the chromosomes indicate the direction of the first maturation division.

12. Equatorial section. The first maturation division is completed and the second maturation division is indicated in some cases.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c. m.
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x
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THE BREATHING AND FEEDING MECHANISM OF THE LAMPREYS. II.

(*Concluded.*)

JEAN DAWSON.

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II. Food and Mechanism of Feeding.

A. *Mode of Attachment.* — If a living *Lampetra wilderi* or an *Ichthyomyzon concolor* (Kirtland) be placed in a glass dish its mode of attachment to the sides of the dish may be observed. The animal, when swimming freely, brings the sides of the oral funnel together so that it forms a vertical wedge-shaped cutwater which offers less resistance to the water than the open funnel. When it is about to attach itself, the closed funnel quickly opens against the glass and, with a single rapid backward and forward stroke of the tongue, the animal becomes firmly attached. The tongue in moving forward resumes its normal position in contact with the surface of attachment and is not seen to move again during the time of attachment, unless one attempts to pull the animal away in which case the tongue is seen to move as in the case of

the first attachment. When the lamprey becomes accustomed to being handled it will seize one's finger and a strong suction may be felt resulting from the action of the tongue in the process of attachment.

If the oral funnel of a recently dead lamprey be pressed against the dish or finger, it becomes attached. If now one pulls the body backward in a direction at right angles to the surface of the attachment, it is found to be firmly attached. This attachment is so firm that one may lift from the water a dead lamprey thus adhering to the finger. If, instead of pulling the lamprey backward or away from the surface, one pushes it in any direction parallel to the plane of attachment, the oral funnel glides easily over the surface of attachment. When the lamprey is attached to a moving fish, the weight of the body as it drags through the water exerts a backward pull on the lamprey in spite of which the animal is able to maintain its hold, though at the same time it is free to glide in any direction over its host.

Since the dead lamprey remains attached, it is clear that the maintenance of the vacuum which effects the attachment is not through muscular action, but through mechanical means. Thus it is probable that the living lamprey may at times remain attached to the host without the expenditure of any muscular energy.

Is the vacuum by which the animal maintains its hold formed in the mouth cavity alone or in both mouth and pharynx? To determine this, the following experiments were tried on *Lampetra wilderi*. (1) A capillary glass tube was fitted over a cambric needle so as to form a small trocar. This was inserted into the nostril of a lamprey and so directed that the needle pierced the dorsal wall of the pharyngeal cavity. The needle was then withdrawn leaving the tube in the opening. The tube was occasionally cleaned by re-inserting the needle. The animal in which a communication was thus established between the pharyngeal cavity and the exterior was able to attach itself as firmly as before, thus showing that the vacuum is formed not in the pharynx but farther forward, *i. e.*, in the mouth cavity.

(2) A glass tube was inserted into the mouth cavity of a second lamprey between the lobes of the tongue. This tube was just long

enough to reach from the mouth opening to a point about 1 cm. back of the semiannularis muscle and establish a connection between the mouth cavity and that of the pharynx. At first the animal made fruitless attempts to attach itself and finally did so as firmly as ever. Upon examination, the animal was found to have swallowed the tube. A tube of the same length, but having a hook at one end, was now inserted into the mouth as before. The hooked end lay between the tongue lobes but did not interfere with the oral funnel in any way. It was found that the lamprey was neither able to swallow the tube nor to attach itself but lay on its side. Finally it was able to attach itself very feebly, but in order to do so the tongue worked back and forward continually, thus creating a slight vacuum.

(3) A guarded bristle was passed through the second external gill opening of a living lamprey into the water tube and on through the mouth so that the enlarged tip of the bristle lay between the tongue lobes, but did not interfere with the action of the funnel. The bristle thus prevented the complete closure of the velar valves and also the close approximation of the lateral tongue lobes. The animal was unable to attach itself except feebly by the aid of the continuous movement of the tongue already described. The bristle was drawn back of the semiannularis muscle so that it could no longer interfere with the action of the tongue, but was left where it would interfere with the action of the velar valves. The animal immediately regained the power to attach itself as firmly as before, thus showing that the velar valves do not aid in forming the vacuum in the mouth cavity.

From these experiments it follows that the partial vacuum which effects the attachment of the lamprey exists in the mouth and oral funnel only. The movement of the tongue at the moment when the animal attaches itself shows that the vacuum is produced at least in part by the pumping action of the tongue. The fact that after the animal has attached itself, the tongue projects forward, shows that the communication between the mouth cavity and the pharynx is not kept closed by the tongue, but probably by the semiannularis muscle, thus leaving the tongue free for other purposes.

In the case of the dead lamprey, the pumping action of the tongue must be replaced by the pressure exerted by the fingers against the oral funnel in the act of attaching the animal, the tongue then remains in the back part of the mouth cavity and thus functions in place of the semiannularis to close the communication between the mouth cavity and the pharynx.

B. *Mode of Feeding of Attached Lampreys.* — This has never been observed, nor is it likely to be, so that our knowledge of the process of feeding is an inference from the structures involved. The moment the animal attaches itself the oral funnel is opened quickly and is pressed flat against the supporting surface. The process may be watched when the lamprey attaches itself to the sides of a glass dish. The funnel may be so much flattened that the teeth on the tongue press against the glass.

In this position the teeth of the oral funnel and tongue constitute a very efficient rasping apparatus. Fürbringer (1875) has shown how the teeth of the funnel are moved in and out on radial lines by the action of the inner layer of the annularis muscle. The arrangement of teeth as already pointed out is such that in their movement along radial lines they necessarily lacerate every part of the surface covered by the funnel. It will be shown below that the tissues of the host are thus reduced to a fine pulp, which is then swallowed.

We may infer that this pulp finds its way into the pharyngeal cavity by the pumping action of the tongue. The considerable size of the pharnygeal cavity (Fig. 4) and the distensibility of its walls enable it to accommodate a large amount of food. By the contraction of the muscular wall, particularly the pharyngeus muscle and the basilaris muscles, the food is forced back into the œsophagus, while the posterior pharyngeus muscle relaxes to permit its passage.

C. *Character of Food.* — As regards the character of the food, there is much difference of opinion. Günther (1853) says: "Die Nahrung des Neunauges besteht ausser Würmern, Insekten, etc. noch in Fischen, sowohl kleinen, als grössern, an welche sie sich wie an Steine festsaugen." Günther does not indicate that he makes this statement from the examination of stomach contents of the lamprey, so that it is possibly merely a current belief.

Abbott (1875, p. 827), speaking of *Petromyzon marinus* says: "This fish which is found occasionally hibernating in the soft mud at the mouths of some of the inflowing creeks appears to come from the bay or ocean (at any rate from the lower portion of the river) in immense numbers early in March and remains about the rocks at the head of tide water for some time as though waiting for the coming shad or herring. With the shad they pass up the river beyond tide-water, and in the rapid rocky portions of the river having deposited their own ova, they wander over the breeding grounds of other fishes and devour every egg they can find. I have found lampreys in Crosswicks Creek in the month of May gathering up the eggs from sun fish nests." But here again the observation is not supported by an examination of stomach contents.

The only other observation known to me is that of Gage (1893) on *P. marinus unicolor*. "Of all the specimens obtained out of the breeding season either the digesting part of the alimentary canal was empty or it contained blood. No partly digested worms or insects or small fish or parts of fish flesh were found, although diligent search was made; consequently it is believed that the lake lamprey is wholly parasitic during its adult life and lives on blood sucked from other fishes." Again (p. 438) he speaks as follows of the intestine at the breeding season: "The atrophy takes place within two weeks, and begins at the terminal extremity, and extends gradually cephalad until the whole canal appears like a thread. As no food is taken during the spawning season there is no necessity for digestion, and in the female there is no room for the intestine when the ova are completely matured."

As it seemed incredible that the very elaborate rasping apparatus of this lamprey could have no other use than to enable the animal to produce a raw surface from which to suck blood, a thorough investigation of all the material of *P. marinus* at hand was made. But in all cases the intestines were found to be empty and atrophied. It was then learned that all the available lampreys had been caught in the breeding season. Through the kindness of Professors Gage and Wilder, however, I obtained several specimens of *Petromyzon marinus unicolor* taken in the

breeding season and one specimen caught in December. The lampreys caught in the breeding season showed the same atrophied condition of the intestine that had been found in our own material, but the intestine of the one that was caught out of the breeding season was found to be gorged with food. The intestine measured 4 cm. in circumference while the intestine of a specimen of the same size caught in the breeding season measured but 6 mm. The œsophagus was entirely empty. Among the contents of the intestine solid particles could easily be seen with the unaided eye, while a microscopic investigation proved most of these to be muscular tissue. The more liquid parts were past recognition. There were recognizable bits of striated muscle about 12 mm. long, a gill and a rib of a small teleost fish. The gill was 1 cm. long and bore filaments 5 mm. in length ; the rib was 2 cm. long. It is impossible to tell whether the lamprey came by this small fish directly or from the intestine of a larger fish which served as its prey.

In any case it appears that the attached *P. marinus unicolor* may feed not only on blood but on more solid tissue. The very extensive injuries produced by this species and figured by Surface (1893) are in accord with this conclusion. Presumably *P. marinus* and *Ichthyomyzon* have similar habits. According to Gage (1893) the adult *Lampetra wilderi* takes no food.

III. Mechanism of Respiration.

A. *When the Lamprey is Attached.* — Respiration may readily be studied when the lamprey is attached, and has been described by Bert (1867), Gage (1893) and Meyer (1835). The respiratory currents may be easily seen in *L. wilderi* and in *Ichthyomyzon concolor* by means of particles suspended in the water. There is a rapidly alternating contraction and expansion of the branchial region. At each contraction a current of water leaves all the external branchiopores simultaneously and passes outward and backward at an angle of 45° with the long axis of the body. At the same time a current issues from the nostril. With the expansion of the branchial region the water is seen entering the external branchiopores in lines converging from all directions to each aperture and at the same time a similar current enters the nasal opening.

Many factors vary the rate of the respiratory movements, such as the vitality of the individual animal, temperature and the oxygen content of the water.

The movement when the animal is attached is regular unless foreign particles get into the gills, in which case spasmodic contractions of the gills take place in order to expel the irritating particles. These contractions are essentially the same as in regular respiration except that they are longer continued and stronger, so that the external branchiopores are brought closer to the water tube. If many solid particles are suspended in the water the rate of breathing becomes slower and may even stop for a full minute, but if the animal is removed to clean water breathing again becomes normal.

During both expiration and inspiration the external branchiopores are wide open and look like so many gaping, round mouths. They do not change their form in either inspiration or expiration. The ectal valves are not taut; with the aid of a lens the ectal valves may be seen flapping idly back and forth in the opening (Fig. 10, *a*). With each inspiration the ental valves are seen to be swept back into the gill pouch much as swinging doors might be. At each expiration the out-flowing water sweeps the ental valves forward and out through the external gill opening past the loose border of the ectal valve (Fig. 10, *c*). It is thus clear that in the normal respiration of the attached lamprey the valves of the external branchiopores have no office other than to perhaps aid in directing the out-flowing water backward.

The movements of respiration and the working of the ental and ectal valves could be seen somewhat more plainly in *Ichthyomyzon concolor* than in *L. wilderi*. Besides the regular respiratory movements recorded for *L. wilderi* a slight backward and forward motion of the branchial basket was observed.

From the arrangement of the muscles of the gill sacs and gill pouches it may be inferred that the expulsion of the water is brought about by contraction in all directions of the lumen of the gill sac, while at the same time the long axis of the sac is more shortened than any of its other dimensions. Thus the capacity of the gill sac is reduced and a part of the water is ex-

pelled. The lateral end of the muscular pouch carries inward with it the elastic branchial basket to which it is attached. When the muscles relax, the elasticity of the branchial basket serves to elongate the gill sacs and thus to fill them again with water. The action may be likened to that of the mammalian lung in which the lung is filled by muscular action and emptied chiefly by the elasticity of the thoracic walls and lungs. The gill sacs of the lamprey are, on the other hand, filled by the elasticity of the branchial basket and emptied by muscular action. Not all the air in the lungs is changed by a single respiration and not all the water in the gill sacs can be changed at a single respiratory movement. The expulsion of the water at the angle of 45° does not retard but rather aids the progressive movement of the fish, while it permits the external branchiopores to have such a form and point in such a direction that they offer the minimum resistance to the movement of the animal through the water.

Gage's (1893) statement that the expired water leaves the external branchiopore at a very oblique angle with the long axis of the body while the inspired water enters at an angle of 90° suggests the possibility that during expiration the major axis of the gill sac forms a very oblique angle with the long axis of the body but that during inspiration this angle becomes a right angle. The following experiment was tried to find out whether there was any change in the obliquity of the gills during respiration: A bristle with as large a tip as would enter the external branchiopore was passed through the gill sac into the water tube so that it occupied the major axis of the gill sac. The end of the bristle was left projecting from the external gill opening and formed an angle of 45° with the long axis of the body. This angle remained constant during both inspiration and expiration.

Gage (1893, p. 469) believes this arrangement by which water leaves the gills at a very oblique angle while the inspired water enters at an angle of 90° is a contrivance to prevent the repeated respiration of the same water. The water does indeed leave the gill at a rather oblique angle (45°), but if the observations here made on the current entering the gill openings be correct, the water flows in from all directions much as it enters the mouth of an empty submerged bottle; moreover it is diffi-

cult to see the need of a contrivance to prevent the repeated inspiration of the same water. The expired water is thrown out with great force and to a considerable distance like a stream from a hose. It is thoroughly mixed with the adjacent water which by this means as well as by the movements of inspiration is kept constantly agitated. When we remember the rapidity with which gases diffuse through water thus agitated (Hoppe Seyler, 1896) there can be little doubt that the water inspired by a *Petromyzon* is of practically the same gaseous content as the adjacent water and is unaffected by the expired water. Probably a special contrivance for preventing the repeated inspiration of the same water is little more needed in *Petromyzon* than a similar contrivance for preventing the repeated inspiration of the same air in a mammal.

The current seen issuing from the nostril at each expiration is caused by the compression of the nasal cœcum. The caudal end of this cœcum is bounded above by the cephalic end of the notochord and below by the first two gill pouches (Fig. 1, *f*). As the gill sacs shorten with each expiration the cœcum is pressed against the notochord and water is forced from it. With the lengthening of the gill sac the walls of the cœcum are again separated and water is drawn into it. This current seems to have no other purpose than to bring the water in contact with the olfactory epithelium.

B. *Respiration when the Animal is not Attached.* — The acts of feeding and breathing have now been considered in the normal attached animal, and no use has been found for the velar valves, the velar jaws, the water tube or the valves of the external branchiopore.

When a gill sac expands there is created a negative pressure within it. To relieve this, water must enter the sac either through the internal branchiopore or through the external branchiopore or through both. When the animal is attached water enters necessarily through the external branchiopore alone. When the animal is not attached the mouth is open and water must enter through the external branchiopore, the valves of which are so constructed that they can offer no resistance to an inflowing current, but water may also conceivably enter the mouth, pass the velar valves and enter the gill sacs by way of the water

tube and internal branchiopores. Thus both when the animal is attached and when it is not attached, water must enter the external branchiopores during inspiration. When the animal is not attached the water thus inspired through the external branchiopore may possibly be mixed in the gill sac with water inspired through the mouth. Indeed the inspiration of water through the mouth may be one means by which the unattached lamprey secures food. It is generally believed among writers (Mayer, 1835; Gage, 1893; Couvreur, 1897) that water may be inspired through the mouth of the lamprey when unattached. It is possible that breeding lampreys behave differently in this respect from those that are not breeding, but none of these authors state the time of the year in which their observations were made.

In order to ascertain whether water is normally inspired through the mouth, the following experiment was tried. A breeding *Lampetra wilderi* was placed in a wire basket which was in turn immersed in a dish of water. The meshes of the basket were small enough to keep the animal from getting out, but afforded no surface to which the animal could attach itself when it came to rest. A carmine mixture was placed near the mouth of the animal when it was thus quiet and unattached, but no current of water could be seen passing into the mouth. Many trials were made, but with the same result. This led me to the conclusion that in the adult lamprey, of this species at least, no current of water is taken into the mouth, but this is of course not true of the larval form.

On November 29, 1904, two living specimens of *Ichthyomyzon concolor* (Kirt), were obtained from the Detroit river and placed in a large aquarium. When one of these was placed on its back without unduly exciting it, the animal immediately became quiet and after a short time, while it remained in this position, could be handled as though it were dead. As soon as it assumed its normal position, however, the animal became active again. The phenomenon seemed to be of a hypnotic nature and obviated the necessity of giving chlorotone to quiet the animal.

While the animal lay on its back a mixture of carmine and water was poured into the upturned oral funnel. Now and again a red current of water could be seen passing into the

mouth and out through the external branchiopores. After a few inhalations, the current would be reversed and a stream of water would be sent out of the mouth with some violence as though the carmine in the water was found to be irritating. The current of water passing into the mouth, it will be remembered, was not seen in the *L. wilderi*.

The two specimens were kept alive for five weeks in the laboratory when they died after being attacked by fungus. During their life in the aquarium, they were not observed to have taken any food, although large pieces of raw beef were suspended in the aquarium.

The only advantage to the lamprey of an inspired current through the mouth would lie in the fact that by means of such a current food might be caused to enter the mouth when the animal was free. The experiment on the inspiration of water through the mouth in *L. wilderi* above recorded was necessarily performed during the breeding season when the brook lamprey takes no food. It is therefore inconclusive. The similar experiment on *I. concolor* performed out of breeding season would tend to support the statement of Günther (1853) and Abbott (1875) that the lampreys feed when free as such feeding could hardly be accomplished without the inspired current through the mouth.

C. *Detachment and Regurgitation.*—The valves of the external branchiopores are clearly useless in aiding the animal to draw water into the mouth. There remains but one use for these structures, namely, to render possible an expired current through the mouth.

When the lamprey is firmly attached there is a partial vacuum in the mouth cavity and the oral hood. It would require considerable force for the animal to tear itself free when thus attached, but if by closing the external branchiopore and opening the velar valves and mouth it can force water into the oral funnel from the gill sacs, the vacuum will be at once destroyed and the animal may free itself without great muscular exertion. There is a further possible advantage to the animal in being able to expel a current of water from the gill sacs through the mouth. Such a current would enable it to cleanse the pharynx and mouth of indigestible and bulky food particles, nor does there appear to be any other means of accomplishing this.

In order to learn how the animal detaches itself the following observations were made : The ectal and ental valves of the external branchiopore were watched very closely with a lens to determine their action, if any, at the moment when the animal detached itself. This proved to be no easy task since the valves were found to act so quickly that one could see but a flash of white in the dark gill opening and the animal was gone before one could see what had happened. This difficulty was greatly lessened by the use of a small amount of chloretone in the water. This increased the rate of breathing at first but then gradually lowered it so that one could more easily tell what was taking place.

The cephalic and caudal sides of the gill opening were seen to be approximated so that instead of remaining circular, as during ordinary respiration the opening became elliptical with its longest axis dorso-ventral. Thus the ectal valve was stretched taut. The ental valves could be seen to come together so as to close this gill opening. Almost the instant the valves closed, the animal detached itself.

It thus appears that the valves of the external branchiopore, act in the manner indicated by their structure, to close the branchiopore at the moment when the animal detaches itself. Experiments were now tried to see if the water was sent to the mouth cavity to destroy the vacuum as it seemed from a knowledge of the workings of the valves and their action at the moment of detachment of the animal that it might be.

Experiment 1.—The head of a lamprey while attached to the side of a glass dish was gently pushed up out of the water without detaching the animal. Care was taken to thoroughly dry the the glass around the oral funnel. When the animal detached itself, water was seen to run down the side of the dish from the mouth.

If the lamprey's head and several gills were thus lifted above the water, air bubbles and water are expelled from the mouth upon the animals detaching itself. The air bubbles must have been taken in through the gill openings which were above the water and must have passed forward through the water tube and pharynx into the oral funnel.

Experiment 2.—A thin mixture of carmine and water was introduced into a gill of a *L. wilderi* attached to the side of a glass dish, by placing the end of a pipette over the external branchiopore and gently but steadily pressing the bulb. The animal expelled the carmine from the gill opening with violence. When a thicker mixture was used, the animal made a great effort to expel it, but when the carmine was still steadily poured into the gill, the animal detached itself and a stream of red was seen to issue from the mouth. When carmine was seen to issue from the mouth, no carmine issued from the gills, thus showing that the valves were acting to close the external branchiopore and to cause the water to take a forward course so as to make detachment easier for the lamprey. These experiments seem to leave no doubt that detachment is effected in *Lampetra wilderi* by a current of water forced from the gill sacs into the mouth cavity and that this is rendered possible by the closure of the external branchiopore by its valves.

As far as I have been able to find, the valves of the external branchiopore are mentioned by but two writers.

Mayer (1835) speaks of two flaps at the external branchiopore and describes them as being swept out and in through the branchiopore by the respiratory current. He assigns no function to them except that of forming a tube for the outflowing water.

Gage (1893) says: "In the case of the lamprey one might think at first that no valves were necessary in respiration, for if the branchial pouches are open to the surrounding medium through the branchiopores any enlargement of the branchial spaces would cause the water to enter, and conversely, any constriction would empty the branchial sacs. This view is correct but this mode of simply drawing water into a sac and expelling it has not apparently answered the requirements of the lamprey, and there is present the thin valve (the ectal valve) which covers the entire branchiopore in the larva, and in addition a double valve (ental valve), which is formed by the growth and modification of the middle gill lamella of the caudal half of the branchial sac." Concerning the function of the valves he says: "In inspiration the two parts of the inner or ental valve turn away from each other and are pressed toward the cephalic wall of the

branchiopore across the channel at the edge of the branchial sac, and the ectal or transverse valve folds over the ental one. By the expansion of the branchial apparatus, the entrance to the gill sac has been rendered more direct and the inflowing stream flows directly into the sac. In inspiration, the water flows through the branchial lamellæ, while around the edges, *i. e.*, at the dorsal and ventro-lateral edges of the gill sac there is formed a canal or gutter by the shortening of the gill lamellæ. The free ends of the lamellæ are also membraneous and curved and aid in making a very complete and smooth canal. The ental valves at the entrance to the branchiopore cross this canal and serve as a guide to the inspiratory stream, not allowing the water to get into the canal around the edges of the gill sac, but directing it into the gill sac itself. In expiration, however, with the change in obliquity and the constriction of the gill sac, the water passes between the branchial lamellæ into the canal and meeting the ental valve rotates the two folds of the valves toward each other and against the caudal wall of the branchiopore, thus removing the obstruction in the canal and really extending it by means of the arched valves. From this arrangement it is seen that two distinct objects are attained, the water not only bathes the gills, but passes between the lamellæ, it is then concentrated in a canal with smooth sides where the friction is at a minimum and in its exit from the branchial sac in expiration the valves prevent the used water from making a circle in the gills, and more important, they form a very oblique channel which directs the expiring stream caudad, thus insuring the animal against using the water over and over. In inspiration, on the other hand, from the direction of the opening, the water enters at nearly a right angle to the axis of the animal, and thus fresh or unrespired water is constantly supplied to the gills."

It has been shown above that these valves have another function than the one indicated by Gage. That they may also act to direct the current of water within the gill sac as Gage believes, does not seem to the writer necessarily to follow from the observations on record. The course that water may take within the continuous space of the gill sac, during the inspiration and expiration seems to be determinable only by direct observation or by

experiment, the inflowing stream may indeed be directed toward the center of the gill sac by the ental valve, but when the gill sac begins to empty itself the ental valves respond to the slightest current, the branchiopore is unobstructed and all the water within the gill sac is equally free to pass out through it. It would seem that the water should then flow directly out through the branchiopore rather than that it should take the circuitous course between the gill lamellæ into the smooth channel along the dorsal and ventral borders of the gill sac and thence out.

D. *The Velar Jaws.* — Mayer (37) gives a figure in which the velar jaws are shown, but gives no description of them.

Stannius (1840) speaks of thread-like projections from the cartilage of the velar valves and may thus possibly refer to the velar jaws.

Vogt and Yung (1889) described the velar jaws as a straining apparatus. From the cartilages of the valves "extend five long thin forked points directed forward with their converging ends and thus a strainer is formed which opposes the entrance of bodies from the pharynx."

I have examined the velar jaws of many specimens of *P. marinus* but have never seen them with projections or otherwise than smooth. That the jaws would act as a strainer and serve to hinder the entrance of foreign bodies into the water tube is clear from their position. From the fact that they close when the velar valves close, it is clear that they may seize and hold foreign bodies which are being carried into the water tube. If the velar valves should then open to permit the forward current from the water tube to enter the pharynx, the velar jaws would be opened by the same muscular contraction, the foreign body would be released and swept forward out of the mouth.

Nevertheless pending the examination of stomach contents the question as to what extent, if at all, the lamprey takes food by means of a current of water entering the mouth must be regarded as still open. If food is thus taken, it is quite possible that as food particles are swept past the velar jaws into the water tube the jaws may seize the larger particles, permitting the smaller ones to pass on with the inspired water. The closure of the jaws would be accompanied by the closure of the velar valves and the

stoppage of the inflowing stream of water. If the velar valves were then opened, the jaws would open and the food would be released and if at the same time the œsophagus should open by relaxation of the posterior pharyngeus muscle, the jaws lie so near the opening of the œsophagus that the food with a small quantity of water would be swept into the œsophagus. The more one considers the mechanism, the more does the conviction deepen that the lamprey is able to feed by means of a water current through the mouth and by the aid of the velar jaw.

Summary.

1. When *Lampetra wilderi* is swimming, the sides of the oral funnel are approximated so that the opening into it is reduced to a narrow vertical slit, fringed by the oral cirri. The compressed oral funnel then serves as a vertical wedge-shaped cut-water.

2. When *Lampetra wilderi* is about to attach itself to any surface, the oral funnel is spread and applied to the surface with a quick backward and forward movement of the tongue and the animal becomes attached.

3. If an opening is made between the pharyngeal cavity and the exterior in *Lampetra wilderi*, the animal is still able to attach itself in the same manner as though uninjured.

4. If a communication be established between the mouth cavity or cavity of the oral funnel and the pharyngeal cavity or the exterior, the animal is unable to attach itself.

5. Attachment in *Lampetra wilderi* is therefore effected by means of a partial vacuum created in the cavities of the oral funnel and mouth by piston-like action of the tongue.

6. A dead *Lampetra wilderi* becomes firmly attached when its oral funnel is pressed against a surface with the fingers, and remains thus attached.

7. It follows from six that an attached *Lampetra wilderi* does not necessarily exert muscular energy to maintain its hold.

8. The oral funnel of a dead or living *Lampetra wilderi* may be moved about freely and very easily over the surface to which it is attached so that the animal is enabled to glide over the surface of its host and so change its position with ease.

9. The oral funnel of a dead or living *Lampetra wilderi* may

be pulled at right angles from the surface to which it is attached only by the exertion of considerable force.

10. It follows from 9 that *Lampetra wilderi* attached in a current of water may retain its hold without necessarily exerting muscular energy.

11. In inspiration in the attached *Lampetra wilderi*, water enters each external branchiopore and the nasal tube, coming gently from all directions, just as it enters the mouth of an empty, submerged bottle. It does not enter merely at an angle of 90° to the long axis of the body as stated by Gage for *Petromyzon unicolor*.

12. In expiration water leaves each external branchiopore in a backwardly directed stream which forms an angle of 45° with the long axis of the body. At the same time a stream issues from the nasal sac.

13. No muscular mechanism has been found to account for the expansion of the gill sacs by means of which inspiration is effected and the inpiration is therefore attributable to the elasticity of the cartilaginous branchial basket.

14. During expiration the gill sacs are compressed and their long axis shortened by the action of the following muscles: *a*, the internal and external compressors of the gill sac; *b*, the superficial compressors of the gill pouch (a muscular pouch which encloses each gill sac and is separated from it only by a large lymph space).

15. Water is forced out of the nasal opening at each expiration by the compression of the nasal cœcum between the notochord and the adjacent first and second gill pouches.

16. Water is drawn into the nasal opening at each inspiration by the expansion of the nasal cœcum due to its attachment to the adjacent medial walls of the second and third gill pouches which are separated by the elongation of the axis of these pouches during inspiration.

17. During both inspiration and expiration the external branchiopore has approximately circular form and the valves guarding it (ectal and ental valves of Gage) are seen to flap idly in and out of the opening.

18. The ectal valves may, as claimed by Gage, help to direct the inflowing current of water to the central part of the gill sac,

but the writer has obtained no evidence that this is the case, nor does she see any reason to believe that during expiration there is a movement of the water within the gill sac in the definite manner indicated by Gage.

19. In the attached *Lampetra wilderi* and *Ichthyomyzon concolor* in normal respiration, the ectal valve of Gage is relaxed, that is, its free edge is not stretched and no other function is observable in it than that of aiding in directing the expired current of water.

20. At the moment when *Lampetra wilderi* or *Ichthyomyzon concolor* detaches itself the dorso-ventral axis of the external branchiopores is seen to elongate so that the free border of the ectal valve is stretched taut. The ental valve at the same time strikes against the inner surface of the ectal valve and is thus prevented from turning outward. The external branchiopore is thus closed.

21. The elongation of the dorso-ventral axis of the external branchiopore is due to the contraction of the muscles, the ectal and ental, which act upon the cartilaginous ring to which the valves are attached.

22. If the head of a *Lampetra wilderi* is above the surface of the water, at the moment of detachment, a few drops of water are seen to issue from the oral funnel. If the head of an attached lamprey is far enough above the surface of the water so that two or more branchiopores are exposed, air and water issue from the mouth when the animal detaches itself. If carmine laden water has been introduced into the gill sac of an attached animal the head of which is submerged, a red stream is seen to issue from the mouth at the moment of detachment.

23. It is concluded from 20, 21 and 22, that detachment is effected by a current of water directed forward from the gill sacs through the water tube and pharynx so as to destroy the vacuum in the mouth and oral funnel.

24. If a thin mixture of carmine in water be introduced into the gill sac of a free *Lampetra wilderi* or *I. concolor* by means of a pipette held opposite the external branchiopores, the fluid is expelled from the external branchiopores by contraction of the gill sacs somewhat more violent than those of ordinary respiration.

25. If a thick carmine mixture be introduced as under 24 it

frequently happens that a violent contraction of the branchial region follows, accompanied by a discharge of the carmine from the mouth. At the moment of this discharge the external branchiopores are seen to be closed as noted under 20.

26. If a stream of thick carmine mixture be directed gently into the oral funnel of a free *Lampetra wilderi* or *Ichthyomyzon concolor* so as to fill the funnel, it frequently happens that there is a violent contraction of the branchial sac, accompanied by a stream of water which issues from the mouth and expels the carmine solution. At the same time the external branchiopores are seen to be closed as noted in 20.

27. It is concluded from 25 and 26 that a current of water from the gill sacs forward through the water tube, pharynx and mouth cavities is the agent by means of which these lampreys habitually cleanse pharyngeal and mouth cavities and is the only means by which bodies too large to pass into the alimentary canal or through the branchiopores, may be removed.

28. The current of water directed forward through the mouth, by means of which these lampreys are able to detach themselves and to cleanse the mouth cavity and pharyngeal cavity affords the first adequate explanation of the function of the valves of the external branchiopore, of the water tube and of the velar valve.

29. In the free *Ichthyomyzon concolor*, by the use of carmine and water, a gentle current may sometimes be seen entering the mouth during the inspiration and passing out through the gills.

30. The teeth of the oral funnel of *Petromyzon marinus* are so arranged in concentric loops that when moved in radial lines by the action of the annularis muscle they lacerate every part of the surface with which the funnel is in contact.

31. The tongue of the attached *Lampetra wilderi* or *Ichthyomyzon concolor* may be thrust forward so as to bring its teeth in contact with the surface of its host and thus when the animal is feeding the lingual teeth aid those of the oral funnel in lacerating the tissue of the host.

32. When the tongue is thrust forward in these attached lampreys so that it no longer serves to maintain the vacuum in the mouth cavity by closing the cavity posteriorly, the semiannularis muscle is believed to contract and thus to maintain the closure

of the mouth cavity. In this way the lamprey is enabled to use the tongue in feeding without loosening its hold on its host.

33. After relaxation of the semiannularis muscle the food in the oral hood of the attached lamprey is believed to be pumped into the pharynx by the piston-like action of the tongue working in the mouth cavity and to be forced thence into the œsophagus by the contraction of the muscles of the pharyngeal wall.

34. The intestine of a single specimen of *Petromyzon marinus unicolor* taken in December from Cayuga Lake, N. Y., was found to contain not only blood but muscle, bone, the gill arch of a small teleost and other tissues, probably those of the host to which the animal had been attached. Gage's ('93) statement that this species feeds only on blood of the host is thus erroneous.

35. In *P. marinus*, *Lampetra wilderi*, and *Ichyomyzon concolor* there projects forward into the pharynx from the cephalic end of the united walls of the œsophagus and water tube a pair of jaw-like structures, the velar jaws.

36. The velar jaws are adjacent to the velar valves which guard the opening from the pharynx into the water tube and the two are supported by a continuous pincer-shaped cartilaginous frame work, actuated by muscles in such a way that closure of the velar valves approximates the velar jaws; while opening of the velar valves separates the velar jaws.

37. When the attached lamprey is feeding, the velar valves close the entrance of the water tube and thus prevent food from entering the water tube and gill sacs. The velar jaws are closed and it is believed passive during this process.

38. The statement made by Günther (1853) and Abbott (1875) to the effect that the *free* lamprey feeds on fish and eggs or small invertebrates are not supported by the examination of stomach contents.

39. It may be possible for the lamprey to feed when free on minute forms or on somewhat larger animals siezed and held between the approximated halves of the oral funnel. The minute forms or the fragments resulting from the laceration of the larger forms might then be carried to the pharynx by a current of water entering the mouth.

40. If the lamprey feeds when free by means of a current of water entering the mouth it is possible that by a simultaneous closure of velar valves and velar jaws, food particles which would otherwise be swept into the water tube are held between the velar jaws. By the simultaneous opening of the velar valves and velar jaws a current of water from the mouth cavity into the pharynx might carry a food particle from the velar jaws into the open œsophagus. On the other hand, a current of water from the water tube might expel such a food particle through the mouth.

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THE CRANIAL AND SPINAL GANGLIA AND THE VISCERO-MOTOR ROOTS IN AMPHIOXUS.

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As my study of the central nervous system of *Amphioxus* must be interrupted for some months on account of other work, I will publish now a brief description of the cells of origin of the nerve components which constitute the dorsal roots.

Reviews of the literature on the nervous system of *Amphioxus* are given in papers cited below (nos. 3, 4, 5,) so that it will be necessary to speak here only of the work bearing directly on the elements to be described. Rohde (1) thought that the nuclei which he saw in the roots of the dorsal nerves indicated the presence of the equivalent of the spinal ganglia of vertebrates. On p. 199 he says: "Allenthalben liegen den sensiblen Nerven Kerne eingebettet, welche namentlich häufig bei ihrem Abgange vom Rückenmark auftreten. Sie haben genau dasselbe Aussehen wie die besonders in der Epithellage des Hirnventrikels häufig vorkommenden Nervenkerne und sind diesen sicherlich identisch, also nervöser Natur . . . Den Spinalganglien der höheren Wirbelthiere entspricht also bei *Amphioxus* eine Ansammlung nervöser Kerne." The author then reviews earlier comments on these nuclei. Neither Rohde nor earlier authors saw cell bodies or nerve processes belonging to them. Evidently the mass of nuclei described by Rohde is continuous with the small nests of ganglion cells mentioned by Hatschek (2). This author says: "Die dorsale Wurzel, welche bekanntlich keine Verbindung mit der ventralen eingeht, steigt nahezu in dem Winkel des Myoseptums gegen die Unterhaut empor und theilt sich dort in einen dorsalen und ventralen Ast. Kleine Nester von Ganglienzellen finden sich besonders an der Theilungsstelle des Nerven, z. T. aber auch schon in dem aufsteigenden Teile und auch in den Ästen. Der aufsteigene Teil ist daher als eine ausgezogene Wurzel zu betrachten und die Spinalganglien, welche wenig konzentriert sind, liegen in der Unterhaut (in unmittelbar Nahe

ihres epithelialen Entstehungsortes)." The methods used by these authors were inadequate to demonstrate the character of the cells to which they called attention and later authors working by special methods have denied the nervous nature of these cells.

Retzius (3) describes two types of cells within the spinal cord of *Amphioxus* which send fibers out in the dorsal roots. One type consists of small and medium sized bipolar cells transversely placed at either side of the central canal or extending across it. From one end of the cell a fiber enters the dorsal root. The second type consists of longitudinally placed bipolar cells from either end of which a fiber passes rostrad or caudad in the dorsal fiber bundles. One of the fibers arising from such a cell divides in T-form, sending a lateral branch into the dorsal root. After reviewing the relations of these cells Retzius says (p. 45): "Wo sind nun die *Spinalganglien*? Es giebt deren *keine*. In den sensiblen Wurzeln sucht man sie vergebens, sowohl innerhalb der Rückenmarksgrenze wie ausserhalb derselben. — In dem nächsten Verlauf der sensiblen Zweige trifft man weder einzellne Ganglienzellen noch Gruppe von solchen. Die Asnchwellungen, welche einige Autoren erwähnen, waren gewiss nur zufällige Bildungen. Ebensowenig konnte ich etwaige Stellvertreter, sog. 'Analoga,' der Spinalganglien entdecken; auch die von Rohde in die Wurzeln eingebetteten Kerne, die er geneigt ist als solche zu betrachten, sind meiner Ansicht nach nicht nervös, nicht 'Analoga' der Spinalganglien.

"Wenn also 'Analoga' oder richtiger Homologa der Spinalganglienzellen ausserhalb des Rückenmarks nicht nachweisbar sind, so bleibt die Frage unbeantwortet, ob nicht entsprechende Ganglienzellen im Innern des Rückenmarks vorkommen. Und man hat dann daran zu denken, ob nicht die beiden Reihen longitudinal angeordneter Ganglienzellen, deren Stammfortsätze nach geschehener Teilung (in T-form) in die sensiblen Wurzeln austreten, möglicherweise den Ganglienzellen der Spinalganglien entsprechen können."

Heymans and van der Stricht (4) described the development of the dorsal root by two rootlets, a dorsal cellular and a dorsolateral fibrous strand. With regard to spinal ganglia they say

(p. 16): "Si l'on examine très attentivement des diverses parties constituantes, aux différents stades de leur développment, de cette double ébauche radiculaire, nulle parte on ne constate une trace d'ébauche ganglionaire. L'étude de l'Amphioxus adulte, à l'aide de la méthode de Golgi, à l'aide du bleu de méthylène et à l'aide des méthodes ordinaires, ne révèle l'existence d'aucune cellule ganglionnaire proprement dite sur le trajet des racines dorsales, mais dévoile leur présence à l'intérieur du névraxe lui-même. Nous croyons donc pouvoir en conclure qu'il n'existe point de ganglions spinaux ni de ganglions crâniens sur le parcours des racines dorsales chez l'Amphioxus."

Dogiel (5) has described at length certain structures which he finds attached to the nerve rami just distally from the point of division of the dorsal roots into dorsal and ventral rami. The structures appeared in animals subjected for a long time to methylene blue in physiological salt solution. They were never stained by methylene blue dissolved in sea water. Dogiel was uncertain whether they may not have been artificial products due to immersing the animals in physiological salt solution. He was unable to make out their structure in preparations fixed and stained in various ways. He decided that they are normal structures, however, since they stain well with gold chloride and are visible in animals treated with osmic acid. The structures in question are rounded or pear-shaped bodies which are found in clusters of two, three or four attached to the nerve rami as berries are attached by their stems. The author concludes: "Sämtliche aufgezählten Thatsachen lassen sich am ehesten in der Weise auslegen, dass die beschriebenen Elemente Analoga von Spinalganglien darstellen, welche beim Amphioxus möglicherweise in einer embryonalen Entwicklungsform vorhanden sind. Eine endgiltige Entscheidung der Frage über die Natur dieser Gebilde ist jedoch natürlich nur dann möglich, wenn die Structur derselben und ihre Beziehung zu den Nerven sicherer bestimmt sein wird."

I can add with regard to these structures, first that they do stain by methylene blue dissolved in sea-water, and second that they are to be found along the course of the ventral ramus as far ventrally as the middle of the lateral surface of the body.

Although I have had them stained in a large number of animals they have appeared only in specimens which had remained a long time in a relatively strong stain or had been exposed to the air for a long time after staining, or both. In all cases the animals, although alive and reflexly irritable, were in an extreme state of weakness. I have never seen in these bodies stained with methylene-blue such a structure as would indicate that they are normal nervous organs. They always appear as pale, granular or structureless pear-shaped or balloon-shaped bodies attached by the small end to the nerve ramus. I do not see that any fact regarding these bodies suggests comparison with the spinal ganglia of vertebrates. Their form, their position, the fact that they appear stained with methylene-blue only when the animals are subjected to physiological salt solution or are kept in stain in sea water until they have reached a state of extreme weakness, all indicate that they are probably artifacts. From my own observations I should conclude that they are formed by the exudation of fluid from the nerve through a rupture in its sheath. The exuded fluid takes the form of a balloon, remaining attached by a stalk. The most favorable place for the formation of such exudation is in the angle between the dorsal and ventral rami and between two myotomes. They are formed also, but less often, far along the ventral ramus and even relatively far out along the dorsal ramus. If by physiological salt solution in which Dogiel stained his animals, he means a solution of sodium chloride of 0.75 per cent., more or less, this would be very favorable to the formation of such artifacts, since a physiological salt solution *for Amphioxus* must contain upwards of three per cent. of sodium chloride. The animals soon die in salt solutions of less strength. Dogiel states indeed that his animals died quickly in the physiological salt solution and that the structures described were brought to view by the stain only after three or four hours! Maceration must have been going on rapidly during all that time.

Finally, I have been unable to find these bodies in sections of animals well fixed and stained by various methods, or in specimens treated with osmic acid. The resemblance which Dogiel notes between these bodies and certain structures connected with the rostral nerves is not at all close. On the proximal portion

of the first, second, third and fourth nerves of normal living animals are to be seen rounded projections or knobs which might be described as bud-like or as resembling an up-turned thumb. The greatest difference between these structures and the supposed spinal ganglia is that these stain in methylene-blue at the same time with those bodies which are connected with the end branches of the rostral nerves; that is, some hours earlier than the supposed spinal ganglia.

My own observations have been made upon animals stained in methylene-blue and upon sections prepared by the Golgi method and by a variety of hæmatoxylin methods after fixation in twenty per cent. formalin, Worcester's, Zenker's or Flemming's fluid.

In living animals stained with methylene-blue, both types of cells described by Retzius and the fibers from the transverse cells passing into the dorsal roots have been clearly and repeatedly seen. In two specimens also, many examples of slender bipolar cells were seen in the root of the nerve in the situation of the cells shown in Fig. 4. Each of these cells sent one process out along the nerve and one into the cord. When the cells in this position are examined in hæmatoxylin sections they are seen to be spindle-shaped cells with elongated nuclei and with a process from each end of the cell which can be followed for a considerable distance. The cells have very slender bodies with only a thin layer of cytoplasm over the nucleus. Except for the difference in size, which is more or less proportional to the difference in the size of the two animals, these cells are closely similar to the ganglion cells of the cutaneous and lateral line fibers in Petromyzon (*Lampetra Wilderi*, 6). Such cells are found in the root and undivided trunk of the nerve, in the proximal part of the dorsal and ventral rami, and also in the cord near the root of each nerve. The largest number of cells are found in the proximal part of the root and in the somewhat conical protuberance of the cord which gives rise to the root. In the so-called cranial nerves (I., II.) the large size of the roots and the somewhat more regular arrangement of the cells and fibers enable one to see these elements more clearly than in the trunk nerves. Comparison of horizontal and transverse sections shows that in both cranial and trunk nerves the cells are distributed throughout the thickness of the nerve root.

Sections by the rapid Golgi method show these cells and confirm the description given above. Cells of the following types are seen to send fibers into the dorsal roots. In the figures the several types are indicated by the arabic numerals which are used here in the text. (1) Bipolar cells near to or extending across the middle line of the cord, whose central processes go to the further side of the cord from the roots which the peripheral processes enter. The central process after reaching the opposite side of the cord either (*a*) enters the dorsal bundles without dividing, (*b*) divides in T- or Y-form into rostral and caudal branches, or (*c*) ramifies at once more or less profusely. The last mode of behavior is seen most often in my preparations. These cells mingle with the more centrally situated cells of the next type. (2) Bipolar cells within the cord more or less radially placed with reference to the nerve root, whose central processes remain on the same side of the cord as the roots which the peripheral processes enter. These cells may be situated anywhere within the area of a fan whose handle is represented by the nerve root and whose rays are represented by these cells and their processes. The bodies of the cells are situated among the bundles of root fibers as they turn for-

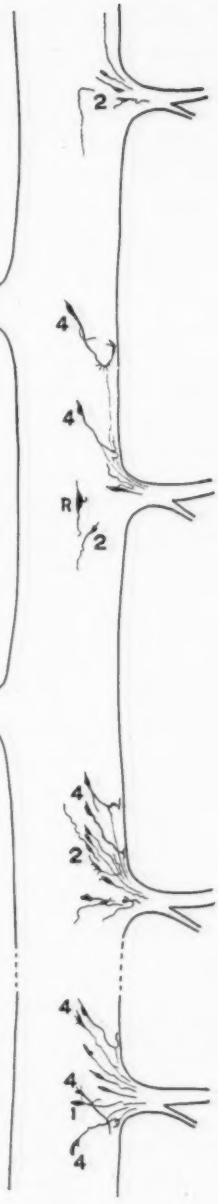

FIG. 1. A horizontal section of the nerve cord and dorsal roots. The parts above and below the dotted lines were drawn from adjacent sections of the same animal. The arabic numerals indicate the types of cells described in the text. *R*, longitudinal bipolar cell of Retzius. The smaller dorsal ramus of each nerve diverges caudally from the larger ventral ramus.

ward or backward in the cord, and the central processes run with the root fibers in the dorsal bundles of the same side. More cells of this type are impregnated than of either of the others but only enough have been drawn in the figures to illu-

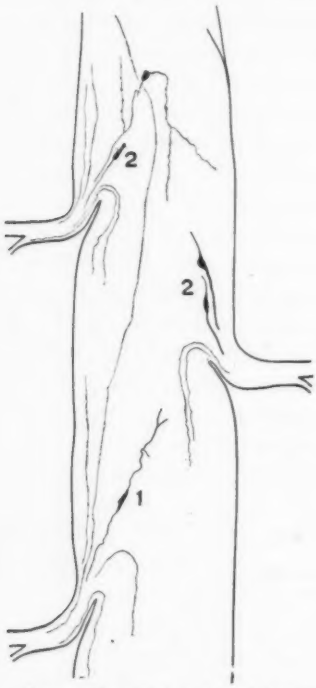

FIG. 2. Horizontal section of the cord between the tenth and eleventh dorsal nerves. The form of the nerve roots shown is characteristic of this region of the body. On the right side are two very coarse fibers with their ganglion cells.

FIG. 3. Horizontal section showing especially fibers going to the opposite side of the cord.

strate their position. (3) Bipolar cells in the root or trunk of the nerve whose central processes are seldom impregnated far into the cord. Those that are impregnated enter the dorsal bundles of the same side. Many root fibers are impregnated which show no cells connected with them. These all run forward or backward in the dorsal bundles of the same side. They

are doubtless fibers whose cells are situated in the root or trunk of the nerve. As shown in Fig. 4, these cells are sometimes present in the dorsal and ventral rami, and it seems probable that they will always be found there. I have not been so fortunate as to have any cells impregnated far out beneath the epidermis in the position indicated by Hatschek, although such cells are readily seen in hæmatoxylin sections. (4) Irregularly pyramidal cells situated near the canal at a slightly more dorsal level than the pigment cells. These cells are usually provided with a single coarse process which runs to the surface of the cord where it ends in a few thick branches or in broad plate-like expansions against the limiting membrane. From some point in its course this thick process (dendrite) gives off a fine fiber which enters the dorsal root. Eight of these cells are shown in Fig. 1.

There can scarcely be any question of the homology of the cells of the first three types described with the spinal ganglion cells of vertebrates. The description confirms the account given by Retzius of cells within the cord sending fibers into the dorsal roots, but it shows that by far the larger number of such cells are situated where Retzius distinctly denied the existence of any nerve cells. They are the cells whose nuclei attracted the attention of Rohde and the earlier authors. The facts given by Retzius together with the discovery (7, 8) that the giant cells in the cord of teleosts are comparable with spinal ganglion cells have been considered as evidence that the spinal ganglia in vertebrates have been derived from the spinal cord. Now that the disposition of the ganglion cells in *Amphioxus* is more fully known it shows that this animal is not so different from vertebrates in this regard as was supposed. In *Amphioxus* the spinal and cranial ganglia form for each nerve an almost continuous mass extending from the central canal of the cord out into the root of the nerve to and beyond the division into dorsal and ventral rami. Thus it may be said that part of the ganglion cells in *Amphioxus* occupy a place within the cord which has been regarded as the hypothetically primitive position for vertebrates, while most of them occupy a place in the nerve roots which approaches the typical position for higher vertebrates. *Amphioxus* is, therefore, not quite primitive in this matter but rather approaches typical vertebrates.

One fact makes it seem probable that there is a movement peripherally of the spinal ganglion cells in *Amphioxus* after the period in the ontogeny when the longitudinal fibers of the cord are formed. Many of the fibers adjacent to the roots are bent out far into the root where they recurve and pass on in their former course within the cord. A slight case of this is shown in Fig. 4. In many cases such recurved fibers extend much further out into the nerve roots, and the number of fibers affected in this way is so great that the bulging laterally of the cord toward each root is very striking in horizontal sections. Occasionally some of the giant fibers are carried toward a root until

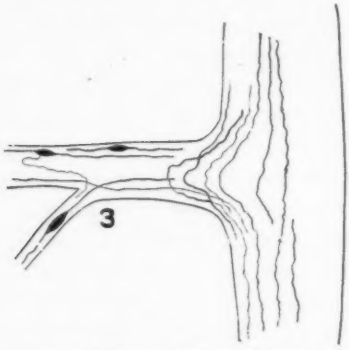

FIG. 4. Horizontal section of dorsal root showing ganglion cells in the trunk and rami. Combined from two sections.

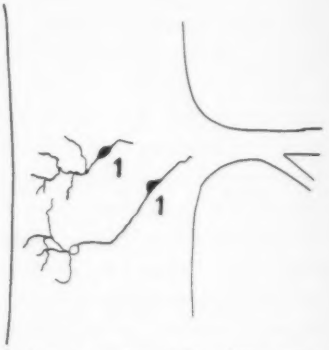

FIG. 5. Two cells whose neurites ramify at once on the opposite side of the cord.

they seem as if they were about to enter it. I can think of no other cause for this curving of fibers out into the roots except the possible active migration of the ganglion cells.

The place of branching of the dorsal roots into dorsal and ventral rami is of some interest. The branching seldom takes place close beneath the dermis as Hatschek describes it. On the other hand, the division of the root near the cord and the separate origin of dorsal and ventral rami directly from the cord are of more frequent occurrence than Rohde states, and are not confined to the anterior end of the body. The separate origin of the rami may be seen in the case of one or several roots in a considerable majority of specimens. Every possible gradation

is to be found between this and the manner of division described by Hatschek. The typical place of division is about half way between the cord and the dermis. This is not only the mean between two extremes, but it is the place where the rami separate in the great majority of cases. In proportion as the division into rami occurs nearer the cord, a greater number of ganglion cells are found in the rami. The argument of Fürbringer (9, p. 646) that the lateral musculature of *Amphioxus* corresponds to the mesial portion only of that of craniates, based upon Hatschek's description of the spinal ganglia of *Amphioxus* outside the muscles, is not supported. The greater part of the spinal ganglion of *Amphioxus* is situated mesial to the muscles as in craniates.

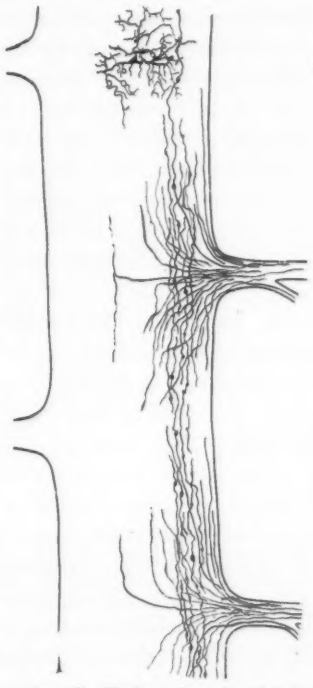

FIG. 6. Horizontal section showing the spreading of the sensory roots into mesial fine-fibered, lateral coarse fibered bundles and the formation of the dorsal compact bundle. The fibers of the last bundle are strongly varicose. At the upper part of the figure is shown the end-branching of a coarse sensory fiber from the root opposite.

It is hoped that the cutaneous and visceral sensory fibers in the dorsal roots may be distinguished, the position of their respective ganglion cells determined, and the central course of each component traced. The more general facts regarding these points seem now to be clear.

The great majority of the fibers of the dorsal roots are fine or medium sized ; a few only are very coarse. The disposition of the fibers in the cord can be seen best in horizontal sections prepared by the Golgi method. As they enter the cord the fibers spread forward and backward and many pass to or across the

middle line, diverging more or less. The fibers which remain on the same side separate into two ill-defined bundles, of which the one nearer the median line consists of the fibers. The medium and coarse fibers are situated laterally. In a high focus, somewhat above the root of the nerve, a distinct bundle of fibers is seen running along dorsal to and independent from the spreading fibers of each nerve. Some fibers of each nerve, however, enter this bundle. The bundle consists of medium coarse fibers

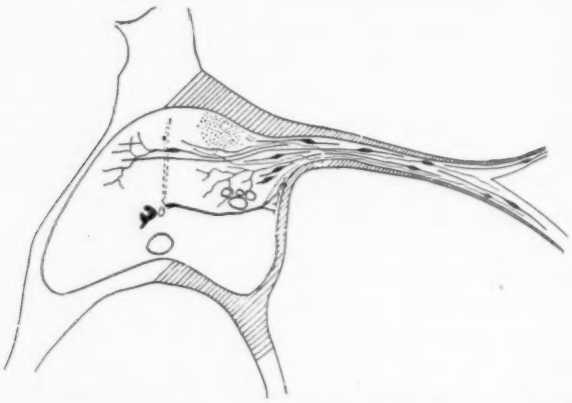

FIG. 7. A diagrammatic transverse section of the nerve cord and a dorsal root. The sheath of the nerve cord is marked with oblique lines. The right lateral group of giant fibers, the mid-ventral fiber and one pigment cell are shown. The stippled area shows the position and size of the compact dorsal bundle of fibers as it appears in the middle region of the body. The disposition of the coarse and fine fibers and their ganglion cells and the position of one viscero-motor cell are shown.

and is situated at the surface of the cord between the mid-dorsal line and the nerve roots. Its position is shown in Figs. 6 and 7. This bundle is distinctly seen in hæmatoxylin sections but has a very different appearance after different fixing agents. It is not well fixed in all fluids. In Zenker's fluid it contracts and the fibers become aggregated into a dense mass which is surrounded by an open space. In twenty per cent. formol the bundle has the appearance of poor fixation with swelling. It appears as a lightly stained reticulated area in which the fibers are not sharply visible. In both these fluids the remainder of the cord seems to be well fixed and stains well. In Worcester's fluid, which has

remarkable penetrating qualities and is a faithful fixing agent, there is no shrinking or swelling and the fibers are well fixed. The bundle appears as an area of rather coarse fibers which take a deeper stain than the remaining fibers in the dorsal region of the cord. The bundle extends throughout the whole length of the nerve cord, at least from nerve II. well into the tail region where it grows very small. The bundle is very noticeably larger on the right side than on the left and on both sides the bundles increase in size toward the head end, perhaps because the majority of the fibers are ascending. In the head region the bundle of the right side is augmented considerably by fibers from each of the nerves VI.–III. inclusive. These fibers run forward and mostly leave the bundle within one segment, for the nerve is small immediately behind each of the nerves mentioned. In brief, the sensory roots form three tracts in the dorsal part of the cord, a diffuse mesial tract of fine fibers, a diffuse lateral tract of coarser fibers and a compact dorsal superficial tract of coarse fibers. The first and second mingle more or less with one another and with fibers of other kinds running ventral to them. The ventral limit of the dorsal tracts taken as a whole is roughly marked by the lateral group of giant fibers.

The very fine fibers seem never to be connected with ganglion cells within the cord, but some of them do have their cells in the nerve trunk. They run for a comparatively long distance in the cord without dividing. Since the visceral sensory fibers in vertebrates are fine, the hypothesis presents itself that these are the visceral fibers in *Amphioxus*. With this their position near the dorsal raphe is consistent. Evidently the visceral fibers do not enter the well defined bundle of coarser fibers; for, if they did, that bundle should be very large on the left side in the head region where all the visceral surface is supplied by nerves of the left side. The bundle is small on the left and larger on the right.

The coarser fibers comport themselves in a variety of ways on entering the cord. (In speaking of coarse and fine fibers of the dorsal roots one must compare the central processes of ganglion cells with central processes and peripheral processes with peripheral processes and in order to do this one must know where the ganglion cell of a given fiber is located.) Speaking then of

the coarser central fibers, some of them ramify at once on entering the cord, others divide in T-form and run in the dorsal bundles of the same side, others enter the dorsal bundles without dividing, others go to the opposite side of the cord and either ramify at once or run in the dorsal bundles, with or without bifurcation. Altogether, the number of coarser fibers which ramify near to the root from which they come is striking. If these are cutaneous fibers a striking physiological fact is explained, namely the relative independence of the segments in locomotion; otherwise expressed, the small number of segments necessary to perform the typical swimming movements. A short piece of the tail end can swim well and behaves much as a whole animal does; and this for many days together.

To illustrate by a complex movement, normal animals in a shallow dish persistently put their heads up over the edge of the dish and then by swimming round the dish and pushing against the edge succeed in wriggling over, if the edge is not too high. The isolated tail makes the same persistent and apparently purposeful efforts when the dish is very nearly full of water. When an animal is so macerated that *all* the tissues except the notochord are gone from the middle of the body, the two parts perform typical swimming movements but each with an independent rhythm. This retention of the power of coördinated movements by a few isolated segments is perhaps connected with the large number of cutaneous fibers which have a short course in the spinal cord. This makes it possible for the muscles to be reflexly controlled by stimuli received at the surface of the body in the same or adjacent segments.

Finally, a few coarse fibers whose ganglion cells are in the nerve trunk go to form the definite bundles described above. Since these are chiefly ascending fibers which have a long course in the cord, the bundle may be compared with the dorsal tract of the same description in vertebrates, the tract of Goll. This is therefore probably the first tract to appear as a definite bundle in the vertebrate nervous system.

The fourth type of cells described in this paper are the viscero-motor cells. In position they correspond to the visceromotor column as it is known in fishes and other vertebrates.

They are lateral to the ventral parts of the canal. They retain primitive characters in that the cell body is adjacent to the canal and that there is a single large dendrite which extends to the periphery of the cord. The origin of the neurite from the dendrite at some distance from the cell body is perhaps also a primitive character so far as the vertebrate nervous system is concerned. With respect to the disposition of these cells along the cord it is evident that they form a more or less complete column and that the neurites often run for some distance in the lateral tracts to reach their roots (Fig. 7).

As our knowledge of the nervous system of *Amphioxus* increases its effect is to oppose the tendency of recent years to minimize the relationship between *Amphioxus* and vertebrates, to consider *Amphioxus* as far removed from typical vertebrates and more closely related to invertebrates rather low in the scale. While there is great significance in the similarity of the nephridium (10) and eye-spots (11) of *Amphioxus* to those of some worms, the close relation of the nervous system of *Amphioxus* to that of vertebrates has also an unquestionable significance. *Amphioxus*, indeed, contributes more toward bridging over the gap between vertebrates and invertebrates than has usually been supposed.

The nervous system of *Amphioxus* agrees with that of lower fishes in the following respects:

(*a*) It is dorsal, hollow, and has separate dorsal and ventral roots of definite composition. The canal has an enlargement at the anterior end, the brain ventricle.

(*b*) The dorsal roots consist of general cutaneous, visceral sensory and visceral motor components. They contain also in the head region fibers of special sense organs (olfactory or gustatory?).

(*c*) Both kinds of sensory fibers have ganglion cells which are situated either within the cord or in the root of the nerve in essentially the same position as the spinal ganglia of vertebrates.

(*d*) The two kinds of sensory fibers on entering the cord form dorsal tracts similar to those in vertebrates. Many cutaneous fibers show the bifurcation characteristic of these fibers in vertebrates.

(*e*) The viscero-motor cells are situated as in vertebrates dorsal to the somatic motor cells, lateral to the ventral part of the canal.

(*f*) The nerve cells retain the position and characters which are typical in the embryos of vertebrates and which are seen in certain parts of the brain of many fishes.

(*g*) The ventral roots arise separately and remain independent. They are true somatic motor nerves.

We have here nothing else than an essentially vertebrate type of nervous system. At the same time there are good indications of a truly primitive, unspecialized condition. In addition to the facts given under *a*, *e* and *f*, there may be mentioned:

(*h*) The total absence of certain specialized structures which characterize all vertebrates, namely, hair cells responding to vibrations in fluid (neuromasts or acustico-lateral organs), and retinal visual organs. (The morphology of olfactory and gustatory sense organs is yet in an uncertain state.)

(*i*) The presence of simple light-percipient organs within the central nervous system, which have apparently been retained from the worm-like ancestors of *Amphioxus*.

(*j*) The very slight development of the brain.

These facts speak eloquently against the supposition that *Amphioxus* is the result of a process of degeneration from any form which had reached a higher degree of specialization, such as selachians or other fishes. Similar facts have been brought forward (6, p. 73) to show the primitive character of the cyclostome brain. We cannot suppose that specialized structures in the nervous system once possessed by the ancestors of *Amphioxus* have been nicely pruned back and reduced in the process of degeneration; nor can we believe that true nephridia and helminthine eye-spots should reappear in a degenerated species whose ancestors in the course of their evolution had lost these very organs. The straightforward interpretation of the nervous system supports the view that *Amphioxus* and Cyclostomes are the lower branches of the vertebrate phylum. [1]

[1] The Worcester's fluid spoken of in the text is as follows:

40 per cent. formalin ..... 10 parts,
Distilled water ..... 90 "
Saturate this with sublimate;
Add glacial acetic to make 10 per cent.

This work has been done at the Smithsonian table in the Naples Zoölogical Station. I wish to acknowledge my great obligation to the Institution for the opportunities thus afforded, and to express my best thanks to the officials of the zoölogical station for the excellent facilities provided.

NAPLES, January 25, 1905.

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PAULINELLA CHROMATOPHORA.

WILLIAM A. KEPNER.

In volume 59 of the *Zeitschrift für wissenschaftliche Zoölogie* Lauterborn has described an interesting new Sarcodin, which he named *Paulinella chromatophora*. During the months of December, 1894, January and February, 1895, he found and studied 200 individuals whose generic and specific characteristics he gave in the following:

"Genus *Paulinella*. Shell elliptical, sack- or flask-shaped, in transverse section circular, composed of five rows of six-sided, silicious plates, mouth of shell elevated upon a neck, very narrow, in transverse section a lengthened oval. The protoplasmic body does not completely fill the shell cavity; nucleus spherical, rather large, with a reticular structure, situated in the posterior part of the body; contractile vacuole in the anterior third of body. Pseudopodia long and slender, never anastomosing.

"*Paulinella chromatophora*. With the characteristics of the genus. Contains one or mostly two conspicuous sausage-formed chromatophore-like bodies of a blue-green color. The reception of food not observed, nutrition, therefore, apparently holophytic with the aid of the 'chromatophores.'

"Length of shells: 0.020–0.030 mm., width: 0.015–0.020 mm., diameter of chromatophores: 0.003 mm.

"Habitat: stagnant water of the Rhein near Neuhofen under diatoms in company with *Amœba*, *Difflugia*, *Euglypha*, *Gromia mutabilis* Bail, etc."

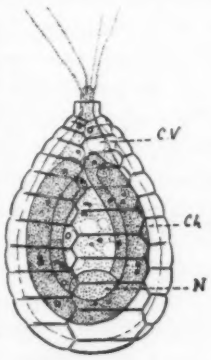
So far as I know this form has not since been discovered. I wish to record it hereby for the United States. The specimens studied met the requirements of Lauterborn's paper so well that I am not justified in giving the details of the anatomy. For these the reader is referred to Lauterborn's account.

Mr. W. G. Lapham, of this place, discovered and recognized, on November 1, 1904, a single individual of *Paulinella chromatophora* in water taken from an oozy bank near Afton, Va. In December the writer found specimens from a spring-pool at Charlottesville, Va. They are mud-loving creatures and are

found in the sediment upon dead leaves in company with many *Mallomonas Plosslii* Perty, some *Amœba radiosa*, and *Acanthocystis* sp. and an occasional desmid.

The shells in our specimens were somewhat thicker than Lauterborn describes.

The bulk of the protoplasmic body varies, but it never fills completely the shell cavity. In one individual the protoplasmic body was contracted into a sharply defined spherical mass, which lay at the base of the shell cavity. Before pseudopodia appear a rounded neck of protoplasm is extended through the narrow neck of the shell. And then suddenly one or more pseudopodia are thrust out from this protruding bit of protoplasm. For a moment the pseudopodia remain quiescent; but they are usually oscillating or slowly waving like a flagellum. It is strikingly interesting to see these pseudopodia function as primitive flagella.



An isolated individual kept in a moist chamber at living-room temperature from January 5 to January 18 showed on the latter date *two large horse-shoe shaped chromatophores* which lay side by side with their ends directed towards the mouth of the shell. On January 5 this individual had but one horse-shoe shaped chromatophore. The specimen lived in this condition one week longer when it was accidentally killed. It remains to be seen, therefore, whether these two large chromatophores were developed as an effect of the artificial environment, or whether they were a step preparatory to cell-division, or a mere variation which will be more frequently met with when greater numbers are found.

We have not counted the individuals found. They are plentiful and can be found with comparative ease in water from the spring-pool by Preston Heights, Charlottesville, Virginia. We have succeeded in keeping *Paulinella chromatophora* alive in small aquaria and moist chambers at living-room temperature for periods as long as three weeks.

UNIVERSITY OF VIRGINIA,
February 11, 1905.

TORSION OF THE CRUSTACEAN LIMB.

FRANCIS H. HERRICK.

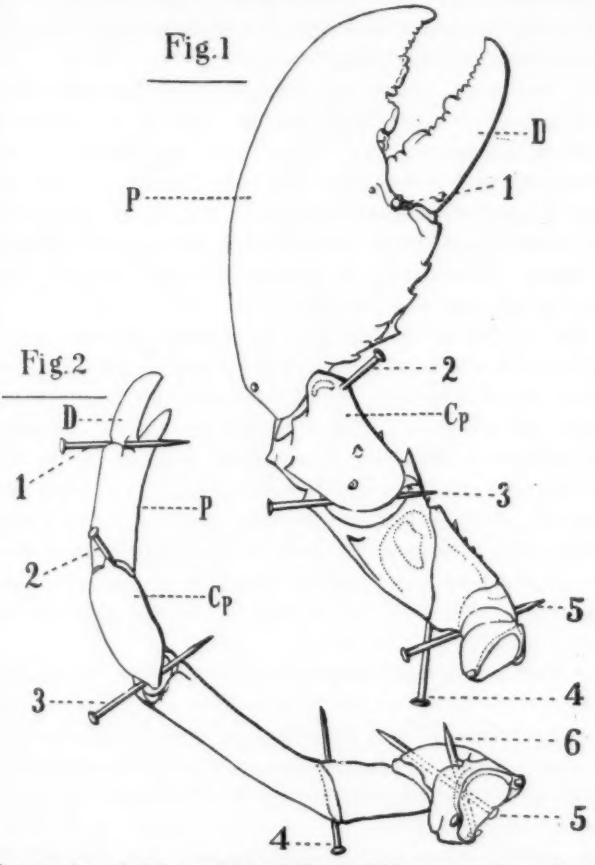
The large double claws or chelæ, which are so conspicuous in many of the higher crustacea, have not failed to attract the attention of naturalists, both on account of their remarkable regenerative phenomena as well as for the aid which they afford to systematic zoölogy. It is therefore the more surprising that attention has never been called to a striking instance of torsion in the great chelipeds of two of the best known forms — the crayfish and the lobster.

If we examine the appendages of either animal from above (Figs. 1 and 2) it will be seen that while in the great claws the dactyles face and open inwards and therefore in a nearly horizontal plane, all the smaller chelæ open upward and outward in a plane which is nearly vertical. In the lobster, however, at the time of birth (Fig. 4) the three pairs of chelate legs, great and small, all have the same form and position, that is, the claws, which are laterally compressed, all open vertically with an inclination outward. It therefore follows that the position of the great 'forceps' has been reversed by rotation through 90°, in consequence of which their inner or anterior faces have become their under sides (Fig 3). The metamorphosis has been reduced to such a degree in the crayfishes that the young are hatched with the essential characteristics of the adult form. If therefore a torsion really occurs in the limbs of these animals, it must be sought in the egg.

In the true crabs, or such representative forms as *Callinectes* and *Carcinas*, the claws open outward, as in the larval lobster, and the outer or posterior face, corresponding to the upper side of the chela of an adult crayfish, tends to become the under side, and is deficient in pigment.

In the crayfish again, about one quarter of the weight of the animal is represented by the great chelipeds, while their proportional weight in the lobster is one half. The acquisition of

size and strength in these limbs, and in *Homarus* the remarkable differentiation into toothed and crushing forceps of either right



FIGS. 1 and 2. Left first- and third chelipeds of *Homarus americanus* in natural position, seen from above. Pins 1–5 are inserted close to the hinge joints of the successive segments in each limb, to illustrate the degree of torsion which the great chelipeds have undergone. Compare the position of the hinge-joints 1 and 2, Fig. 1, with that of the corresponding joints in Fig. 2. *Cp*, Carpodite; *D*, dactyle; *P*, propodite; *1–6*, pins inserted in hinge-joints.

or left sides, has been accompanied by a permanent torsion, which has chiefly affected the carpodite, or fifth joint (as counted

from the base — *c. p.*, Figs. 1 and 2). As I shall later show however, this twisting of the limb is entirely independent of the form or weight of the claw. Meantime the eight slender legs, which follow the larger pair, have remained stationary, and have retained their larval form and position.

In the larval lobster the first pair of chelate limbs are prehensile organs solely, by which the food is seized and transferred to the mouth-parts. Later, when the double claw is fully developed, and is provided with either crushing tubercles, or rows of interlocked tooth-like spines, the prey is usually crushed and torn in pieces before its delivery at the mouth by the smaller claw-feet. The toothed or "quick" claw is primarily used for striking and capturing the prey.

The rotation of the chela, in the lobster, is completed at the fourth molt, which marks the most surprising leap in the whole history of development, for at this time the larval swimming organs are laid aside and as Prentiss * has shown, the antennulary pockets or balancing organs come into play, when, for the first time the miniature lobster swims steadily, and in an upright position. At this crisis new instincts also arise; when the lobsterling swims rapidly at the surface, the great claws are directed forward and held close together, but if its pugnacity is aroused, it assumes the well known attitude of defense displayed by an adult animal.

In dead lobsters or crayfishes the large claws, in response to gravity, lie perfectly flat, but this position cannot be assumed in life unless the muscles of the limb are completely relaxed. In the common-position of wariness and defense, the claws are upraised, and tilted obliquely inwards, so that their tips rest close together on the bottom.

In both *Cambarus* and *Homarus* the disposition of pigment is a more striking index of the rotation of the limb than changes in its form, and even more than in the crab have the under sides or what were once the anterior faces of the claws become differentiated by the absence of color. The mottled green pigments which are so thickly spread over the whole upper surface of these animals are completely lacking on the under sides of the big claws.

* "The Otocyst of Decapod Crustacea: Its Structure, Functions, and Development," *Bull. Mus. Comp. Zoöl.*, Vol. XXXVI., 1901.

The large forceps of either side differs in still other respects from the more primitive type-form preserved in the slender feet. Thus the lower margin of the weaker claw of the small cheliped is incurved, as contrasted with the rotund or convex outlines of the big chelæ, a condition probably determined by the greater muscular development of the latter.

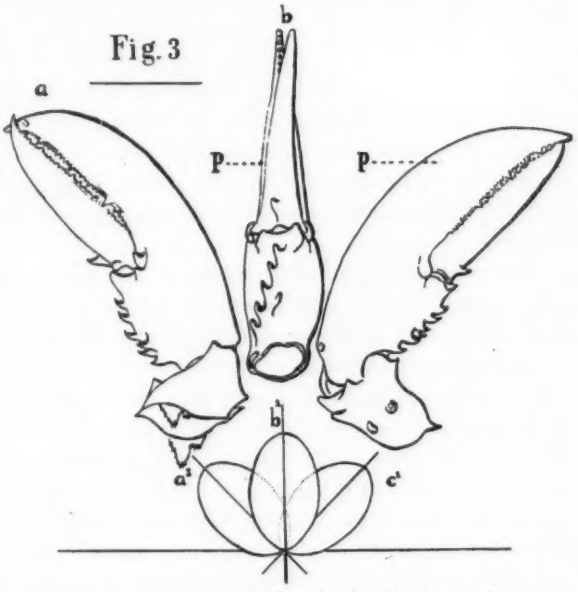


FIG. 3. Illustrating successive positions (a, b, c) in the rotation of the great claws of the lobster, and further by the vertical projection (a^1, b^1, c^1), shown below. In a, a^1, which represents the normal position in the larval lobster and adult crab, the upper or anterior surface of the claw becomes, in the adult lobster the underside c, c^1. P, propodite.

In Figs. 1 and 2, which represent the first and third claw-feet of the left side as seen from above, with the natural foreshortenings, pins are inserted at successive hinge-joints, to illustrate the various degrees of torsion which parts of the limb have undergone. The joints mainly affected are the carpodite and propodite (Cp, P), and the extent to which they have been twisted may be measured by a comparison of pins (or hinge-joints) marked 1 and 2 in the figures. This phenomenon may

be compared in a general way with the remarkable changes which occur in the metamorphosis of the flat-fishes, where the whole body is more or less completely involved. It seems to be confined to the closely allied Homaridæ (*Homarus*, *Nethrops*), Potamobiidæ (*Cambarus*, *Astacus*) and Parastacidæ (*Parastacus Astacoides*) of Huxley, besides the Paguridæ, or hermit crabs, the Galatheidæ,[1] or Spanish lobsters (*Galathea*, *Munida*), embracing many deep sea forms, which with a number of smaller families are often united into the larger division of the Anomura. In all these forms the dactyles of the large claws appear to open inwards, and although in many cases the smaller feet are non-chelate, or the metamorphosis is abbreviated or at present unknown, we may safely assume that their present state has arisen from a primitive condition such as we find illustrated in the first larval stage of *Homarus*.

That the modification in question was of very ancient origin does not admit of doubt. It was already perfected in the Erymoid crustacea which inhabited the Liassic seas, unless naturalists are wholly wrong in assuming that these primitive macroura were the ancestors of the modern crayfishes and lobsters.

According to Huxley[2] forty species of Eryma have been described from the rocks of the middle Lias up to the Jurassic (Purbeck to Inferior Oolite) formations. In *Eryma modestiformis* of Oppel, the claws open inwards, and the stalked eyes are relatively very large, as in an adolescent lobster.[3] After the death of such an individual as Oppel depicts the large claws would lie flat as in a dead lobster, but had no previous torsion in the limbs of this species taken place, the relaxed claw should have turned the dactyles outward as it does in crabs.

It is interesting to find that in the somewhat less primitive *Eryon arctiformis*, from the Jurassic slates of Solenhofen, a very

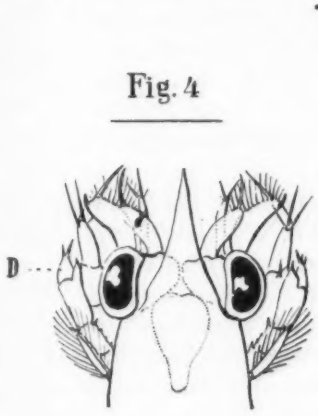
[1] Kinnaman has figured five species of *Galathea*, in all of which, excepting *G. Andrewsii* the large chelæ open inwards, so his drawing is doubtless faulty in this respect. See "A Synopsis of the Britannic Spanish Lobsters and Schrimps," *Proc. Roy. Irish Acad.*, Vol. VIII., Dublin, 1862.

[2] "On the Classification and Distribution of the Crayfishes," *Proc. Zoöl. Soc. London*, 1878, and "An Introduction to the Study of Zoölogy," illustrated by the Crayfish. New York, 1893.

[3] See p. 163 and plate 8, "The American Lobster," Bull. U. S. Fish Commission, Washington, 1895.

decided approach to the brachyurans, or true crabs is seen in the short tail, the broad carapace armed with marginal spines, as well as in the slender antennæ and other appendages so far as they are known. Very significant also is the fact that as in the modern crab the claws open obliquely outward.

In the homarine *Hoploparia*, found in the Cretaceous and early Tertiary periods, the form of the large chelipeds renders it highly probable that a torsion of this limb had already taken place. At all events this phenomenon has a very ancient history, and is probably older than autotomy which precedes the regen-



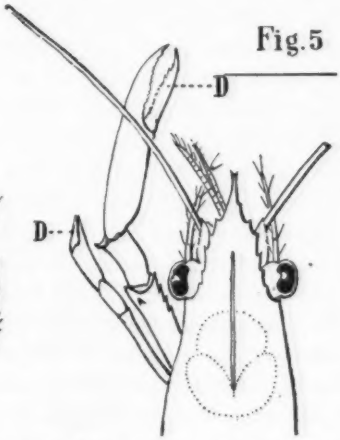


FIG. 4. First larva of lobster from above, showing the outwardly opening claw of the first pair of chelipeds. *D*, dactyle.

FIG. 5. Fourth larva or lobsterling stage of the lobster, showing the big claw-feet, in which torsion is complete, opening inward, toward the middle line of the body. *D*, dactyle.

eration of certain limbs, and which in the most perfect cases is dependent upon a fusion of the second and third joints. In the lobster this fusion does not occur until after the fifth molt.

II.

When we attempt to answer the question — How could a torsion of the crustacean limb be effected? — the results are not very reassuring upon either Lamarckian or Darwinian principles. The

conditions of the problem seem to be very simple, and can at least be stated with a certain degree of exactness.

The segments of the limb mainly affected (Figs. 1 and 2) are the fifth and sixth (*P* and *Cp*, Figs. 1 and 2) which move on hinge joints. Each has a hard tubular shell, and lodges two muscles, a flexor and extensor, the fibers of which originate on the inner surface of the shell, and are inserted on tendons which engage with the next or distal segment, the mobility of the limb being further secured by soft interarticular membranes, and a series of hinge or peg-and-socket joints, set in different planes (Figs. 1 and 2). When the flexor muscle, let us say, of the carpus or fifth joint contracts, the huge claw moves on its hinge and is drawn upward and inward, as a door might be opened by means of cords, worked at a distance: at the same time the fibers react on the inner surface of the tubular shell, but the hinge-joint is fixed, and no conceivable contraction of such fibers can convert a straight pull into a twist. There is no room for the shifting or migration of the muscles, but this would not affect the conditions one way or another. Torsion has not occurred as a result of the normal movements of the limb.

It is to be noticed that the rotation of the claws occurs in larval life before the chelæ attain their full development, but if in the course of the evolution of these forms the increasing weight of the claws could have had any effect upon their ultimate position, it should have turned them in the opposite direction, because they lean outward in the larva. The theory that the effects of strain or use are inherited is therefore incompetent to account for the modification which exists.

In the present case it seems equally impossible to apply the selection theory of Darwin, and it would tax the imaginative faculty to determine wherein the chela of a crayfish was better adapted for prehensile purposes than the claws of hundreds of prawns, or how the toothed claw of the lobster excelled in this respect the pincers of the blue crab, *Callinectes*, or as a weapon of offence, the slashing, sabre-like, out-turned dactyles of *Alpheus heterochelis*, and of many another species of this large genus. I refer only to the power and ease of seizing, and not to the muscular force or to the armature of the claws itself, which is another

question. But the height of the difficulty is not yet reached, for upon the selection theory, the torsion of such limbs must have arisen gradually, through successive fractions of a degree, until the member had moved through a quadrant of arc; furthermore we are required to believe that each successive position was so much more favorable than the last, that forms showing such as well as possibly other correlated variations would outstrip their competitors, and alone leave descendents. As already suggested, the problem is somewhat analogous to the migration of the eye in the metamorphosis of flat-fishes, attempts to explain which in terms of the selection theory have not been very successful.

The difficulty of the problem is not lessened when we reflect that the slaughter of the young in a form like the lobster, takes place on a tremendous scale and is of the most indiscriminate character, before the prehensile organs are fully developed. We can only *guess* at the causes of such a variation. Its great antiquity, dating from as early at least as the Jurassic period, and other considerations already touched upon, render it highly probable that it first arose as a discontinuous variation. That it was not indispensable, is suggested by its erratic distribution at the present day.

Biological Laboratory, Western Reserve University,
February, 1905.

THE PHYSIOLOGY OF LOCOMOTION IN GASTEROPODS.

A Reply to A. J. Carlson.

DR. HERMANN JORDAN,
(University of Zürich).

In No. 2 of Vol. VIII., of this Bulletin, A. J. Carlson publishes a note on locomotion in gasteropods. He, at first, tries to show that my opinion concerning the mechanics of locomotion in *Aplysia*[1] is wrong. On page 87 he says: "Jordan (1901) rejects the theory of 'extensile Muskulatur' in accounting for the locomotion in the marine gasteropod *Aplysia*, and ascribes the relaxation or extension of the longitudinal muscle of the foot to the pressure of isolated bodies of the visceral fluid or blood. As evidence Jordan points to small reservoirs or lakelets of plasma in the strongly contracted foot. These lakelets are constricted off from the visceral cavity by the contraction of the muscular septa. A body of liquid thus cut off from the visceral cavity may serve to produce extension of the longitudinal muscle at its anterior border by the force of contraction of the oblique and transverse muscles at its posterior end. In this way we would have as many isolated bodies of blood being gradually pushed from behind forwards in the foot as there are areas of relaxation on the sole of the foot. The presence of isolated bodies of liquid in the strongly contracted foot is not a sufficient evidence that they are the factors in producing the waves of locomotion, as similar isolated bodies of liquid are also found in the musculature of the contracted mantle (*Aplysia Pleurobranchæa*). . . . Simroth and Jordan missed the true explanation by not taking into account the part played by the musculature of the dorsal and lateral walls of body cavity."

Carlson then describes a mode of locomotion, which he observed only in *Helix dupetithouarsi*. Instead of the ordinary locomotion by waves in the foot, this snail is able to lift up its

[1] *Zeitschr. Biolog.*, XLI., p. 196.

head and to push it forward; it repeats the same movement with the rest of its body, forming in this way two or three waves of the whole body. It is unnecessary to continue the explanation of this mode of locomotion: it is identical with the locomotion in many worms and caterpillars. I say identical, because it is immaterial how many waves there are.

"Nevertheless," Carlson says, at the very end of his note, "the peculiar mode of progression in the snail just described is probably only an exaggerated form of the ordinary locomotion."

I am sorry to be obliged to declare that Carlson did not know or understand my publication at all. Therefore I must repeat the passages in question, and I will try to translate them into English, in order to prevent a second misunderstanding.

Page 199 (*l. c.*) I say: The movement of the foot is formed by waves. To understand this kind of movement we must imagine the sole divided into several parts (Fig. 1). The wave begins through the extension of the first division *AB* in the direction *A*, and by adhering to the ground at *A*. Then the same part contracts, so that *B* approaches *A*. At the same time *BC* leaves the ground, extends, until *B* adheres, then *BC* contracts, *CD* extends and *C* adheres, etc. In this may we have waves advancing — when the foot is lifted from the ground — from the anterior to the posterior part of the body. (In *Helix*, *Limax*, etc., the waves advance in the opposite direction.) All these waves must be combined with appropriate adhesion in order to produce locomotion in the posterior-anterior direction.

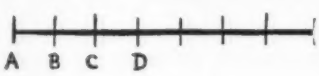


FIG. 1.

The snail is able to change this mode of locomotion spontaneously, *thus, for instance, Aplysia can execute movements similar to caterpillars of geometridæ* (Spannerraupen). I think this explanation should suffice, *for this mode of locomotion is known* (the mode now described by Carlson for *Helix*). In part 3 of the introduction of my work, page 200, I try to explain how extension is possible. This, I think, is the only part that Carlson read; perhaps without understanding it. For in this chapter there is almost no question of waves of locomotion. The whole snail as

well as its single parts is able to extend completely, therefore without coincident contraction of other muscles. This question was studied with pieces of the musculature, and I found that the extension of the relaxed muscle was performed by means of the pressure of the blood. *Under the conditions I observed*, the blood had been pressed into lacunæ beneath the snail's skin (*l. c.*, Pl. II.), and I showed that it was pushed back into the musculature by the extended collaginous tissue, extending it in this way. These lakelets may *sometimes* be completely "isolated."

Concerning the waves of locomotion I say, p. 236: when contraction takes place, water ("blood") is driven into the confined part, which thereby is extended; by this extension tonus is diminished, etc. It will be seen, that I never spoke of "as many isolated bodies of blood being gradually pushed from behind forwards in the foot as there are areas of relaxation on the sole of the foot."

Where is the difference between my opinion and that of Carlson?

Carlson thinks that every extension is produced by blood and organ-pressure, following the contraction of other muscles. According to my opinion, this mode is assumed in normal locomotion, *while the lakelets produce complete or partial extension.* (Perhaps first extension in locomotion also.) Carlson thinks that the special kind of locomotion is nothing else than an exaggerated normal one, consequently also produced by contraction of the "musculature of the dorsal and lateral walls of the body cavity."[1] *This opinion is wrong because, if this musculature is removed, the foot is able to produce normal locomotory waves.*

In conclusion: The opinion Carlson ascribes to me, is in reality not my opinion. My work of 1901 contains all that Carlson publishes as new in 1905, so far as the latter is true. The two modes of locomotion are quite different: in the normal one the blood, which is contained in the lacunæ (not in isolated lakelets!) of the foot, extends the musculature; in the fast one this rôle is performed by the contents of the body cavity, with the aid of the muscles of the dorsal and lateral sides of the body.

ZÜRICH, March, 1905.

[1] This ought to be regarded as the chief result of Carlson's note, because otherwise he would have done nothing to explain *normal* locomotion.